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(12) **United States Patent**  
**Brugliera et al.**(10) **Patent No.: US 6,774,285 B1**  
(45) **Date of Patent: Aug. 10, 2004**(54) **NUCLEIC ACID SEQUENCES ENCODING FLAVONOID 3'-HYDROXYLASE AND METHODS OF ALTERING FLOWER COLOR THEREWITH**(75) Inventors: **Filippa Brugliera**, Preston (AU);  
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C12N 15/29; C12N 15/52; C12N 15/82(52) **U.S. Cl.** ..... **800/298**; 435/320.1; 536/23.2;  
536/23.6; 800/282(58) **Field of Search** ..... 435/69.1, 320.1,  
435/419, 463; 536/23.2, 23.6; 800/282,  
286, 298, 323, 323.1, 323.2, 323.3(56) **References Cited**

## FOREIGN PATENT DOCUMENTS

WO WO 93/20206 11/1992 ..... H05K/7/14

## OTHER PUBLICATIONS

Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410.  
Ashikari, et al. (1989) *Appl. Microbiol. Biotechnol.* 30:515-520.  
Baird, et al. (1987) *EMBO Journal* 6:3223-3231.  
Bechtold, et al. (1993) *Science* 260:1194-1199.  
Bethesda Research Laboratories (1986) "BRLpUC host: *E. Coli* DH5 $\alpha$ <sup>TM</sup> competent cells." *Bethesda Res. Lab. Focus*, 8(2):9.  
Brugliera, et al. (1994) *Plant J.* 5(1):81-92.  
Church, et al. *PNAS USA* 81:1991-1995 (1984).  
Chomczynski, et al. (1987) *Anal. Biochem.* 162:156-159.  
Comai, et al. (1990) *Plant Mol. Biol.* 15:373-381.  
Cornu, et al. (1989) *Petunia Hybrida* 2(14):6.113-6.124.  
Davies, et al. (1993) *Plant Science* 95:67-77.  
D'Alessio, et al. (1992) *Focus* 14:76-79.  
De Greve, et al., *Mol. Appl. Genet* 1:499-511 (1983).  
Dellaporta, et al. (1983) *Plant Mol. Biol. Rep.* 1:19-21.  
Doodeman, et al. (1984) *Appl. Genet.* 67:357-366.  
Dooner, et al. (1991) *Ann. Rev. Genet* 25:173-199.  
Fritsch, et al. (1975) Biosynthesis of cyanidin in cell cultures of *Haplopappus gracillus*. *Phytochemistry*, 14:2437-2442.Frohman, et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002.Gamborg, et al. (1988) *Exp. Cell Res.* 50:151-158.Garfinkel, et al. (1980) *J. Bacteriol.* 144(2):732-743.Forkman, et al. (1980) Anthocyanin biosynthesis on flowers of *Matthiola incana*. Flavanone 3- and flavanoid 3'-hydroxylases. *Z. Naturforsch* 35c:691-695.Forkman, et al. (1981) Genetic control of flavanone 3'-hydroxylase activity and flavanoid 3'-hydroxylase activity in *Antirrhinum majus* (snapdragon). *Z. Naturforsch* 36c:411-416.Forkman, G. (1991) *Plant Breeding* 106:1-26.Franck, et al. (1980) *Cell* 21:285-294.Gleave, A.P. (1992) *Plant Molecular Biology* 20:1203-1207.Guilley, et al. (1982) *Cell* 30:763-773.Hahlbrock, et al. (1979) *Annu. Rev. Plant Physiol.* 30:105-130.Hanahan, D. (1983) *J. Mol. Biol.* 166:557-580.Haughn, et al. (1986) *Molecular and General Genetics* 204:430-434.Holton, et al. (1993) *Nature* 366:276-279.Holton, et al. (1995) *Plant Cell* 7:1071-1083.Inoue, et al. (1990) *Gene* 96:23-28.Ito, et al. (1983) *J. Bacteriol* 153(1):163-168.Jefferson, et al. (1987) *Plant Molecular Biology Reporter* 5(4):387-405.Jefferson, et al. (1987) *EMBO J.* 6:3901-3907.Koorneef, et al. (1982) *Arabidopsis Information Service* 19:113-115.Kozak, M. (1989) *J. Cell Biol.* 108:229-241.Lander, et al. (1987) *Genomics* 121:185-199.Lazo, et al. (1991) *Bio/technology* 9:963-967.Ledger, et al. (1991) *Plant Cell Reports* 10:195-199.Liang, et al. (1993) *Science* 257:967-971.Liang, et al. (1993) *Nucl. Acids Res.* 21:3269-3275.Marchuk, et al. (1990) *Nucl. Acids Res.* 19(5):1154.Markham, et al. *Phytochem.* 34:679-685 (1993).Martin, et al. (1993) *The Molecular biology of flowering.* (Jordan, B.R. ed), UK, CAB International: 219-255.McLean, et al. (1990) *Heredity* 61:341-346.Merrifield, J. (1964) *J. Am. Chem. Soc.* 85:2149-2154.Mizutani, et al. (1993) *Biochem. Biophys. Res. Commun.* 190:875-880.Murashige, et al. (1962) *Physiol. Plant* 15:473-497.Nelson, et al. (1996) "P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature", *Pharmacogenetics*, 6:1-42.Newman, et al. (1994) *Plant Physiol.* 106:1241-1255.Pearson, et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:2444-2448.

(List continued on next page.)

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(57) **ABSTRACT**

The present invention relates generally to nucleic acid sequences encoding flavonoid 3'-hydroxylase (hereinafter referred to as "F3'H") and their use in the manipulation of pigmentation in flowers of plants.

**30 Claims, 25 Drawing Sheets**

OTHER PUBLICATIONS

- Sato, et al. (1997) "Structural analysis of *Arabidopsis thaliana* chromosomes 5.I. Sequence features of the 1.6Mb regions covered by twenty physically assigned P1 clones." *DNA Res.* 4:215-230.
- Schenk, et al. (1972) *Can. J. Bot.* 50:199-204.
- Spribille, et al. (1982) Chalcone synthesis and hydroxylation of flavonoids in 3"-positions with enzyme preparations from flowers of *Dianthus caryophyllus* L. (carnation). *Planta* 155: 176-182.
- Stafford, H.A., (1990) *Flavonoid Metabolism*, CRC Press, Inc. Boca Raton, Florida, USA.
- Stotz, et al. (1982) *Naturforsch* 37c:19-23.
- Tabak, et al. (1978) *Planta* 139:67-71.
- Tanaka, et al. (1988) *Biochem* 103:954-961.
- Tanaka, et al. (1996) *Plant Cell Physiol.* 37(5):711-716.
- Wallroth, et al. (1986) *Mol. Gen. Genet* 202:6-15.
- Wiering, et al. (1984) "Inheritance and Biochemistry of Pigments." In: *Petunia Sink*, K.C. (ed.), Springer-Verlag, Berlin, Germany:49-65.

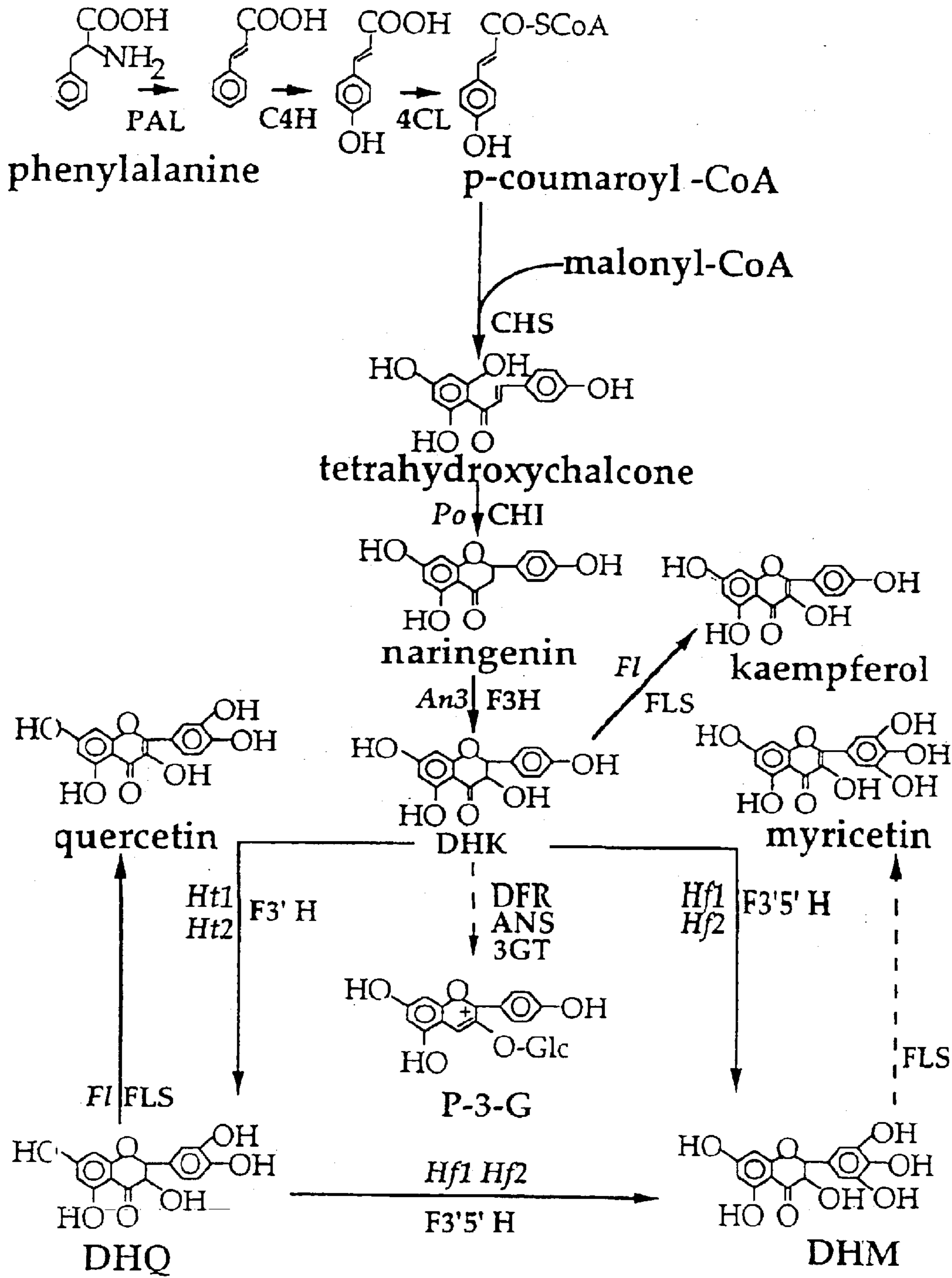


Figure 1a

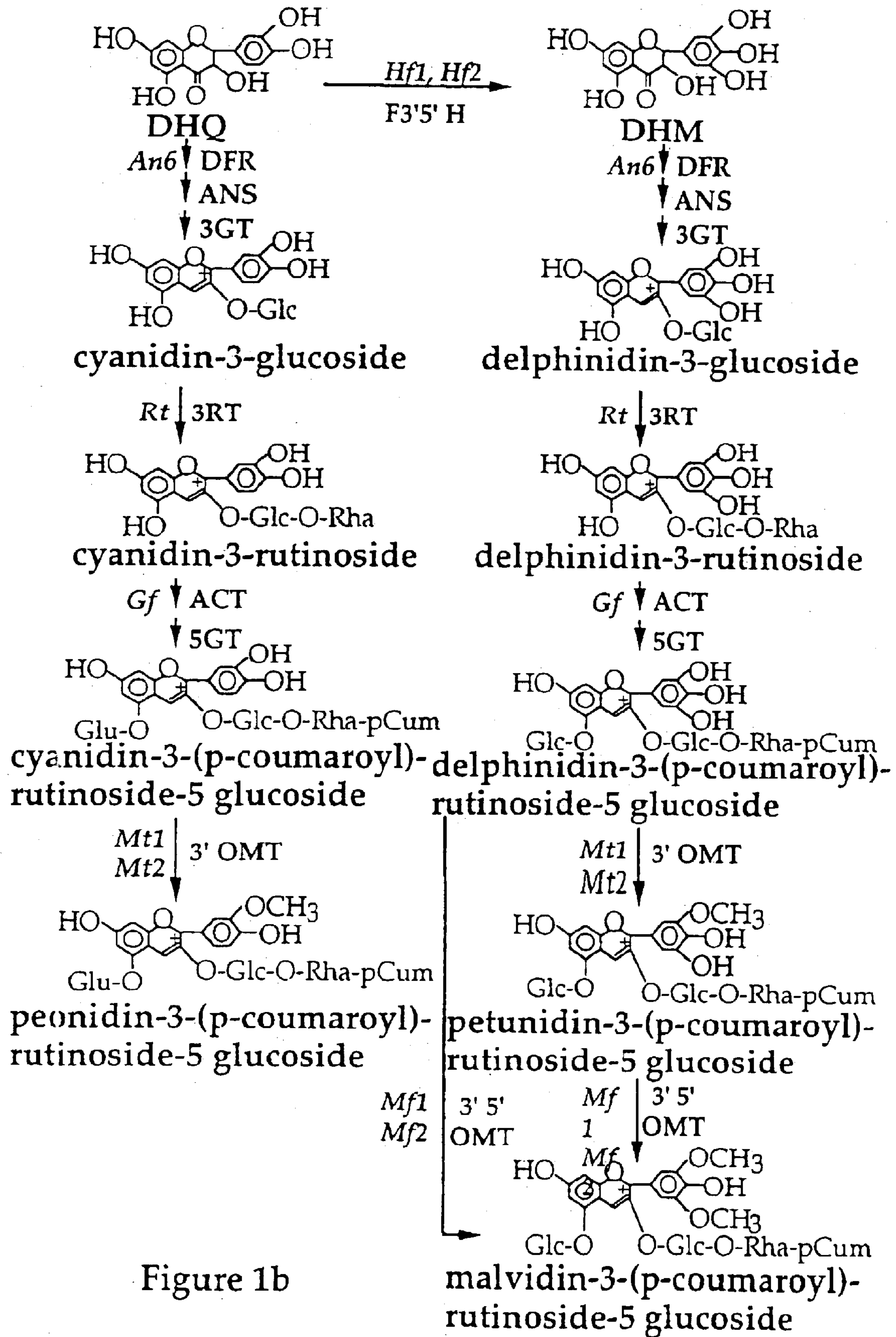


Figure 1b

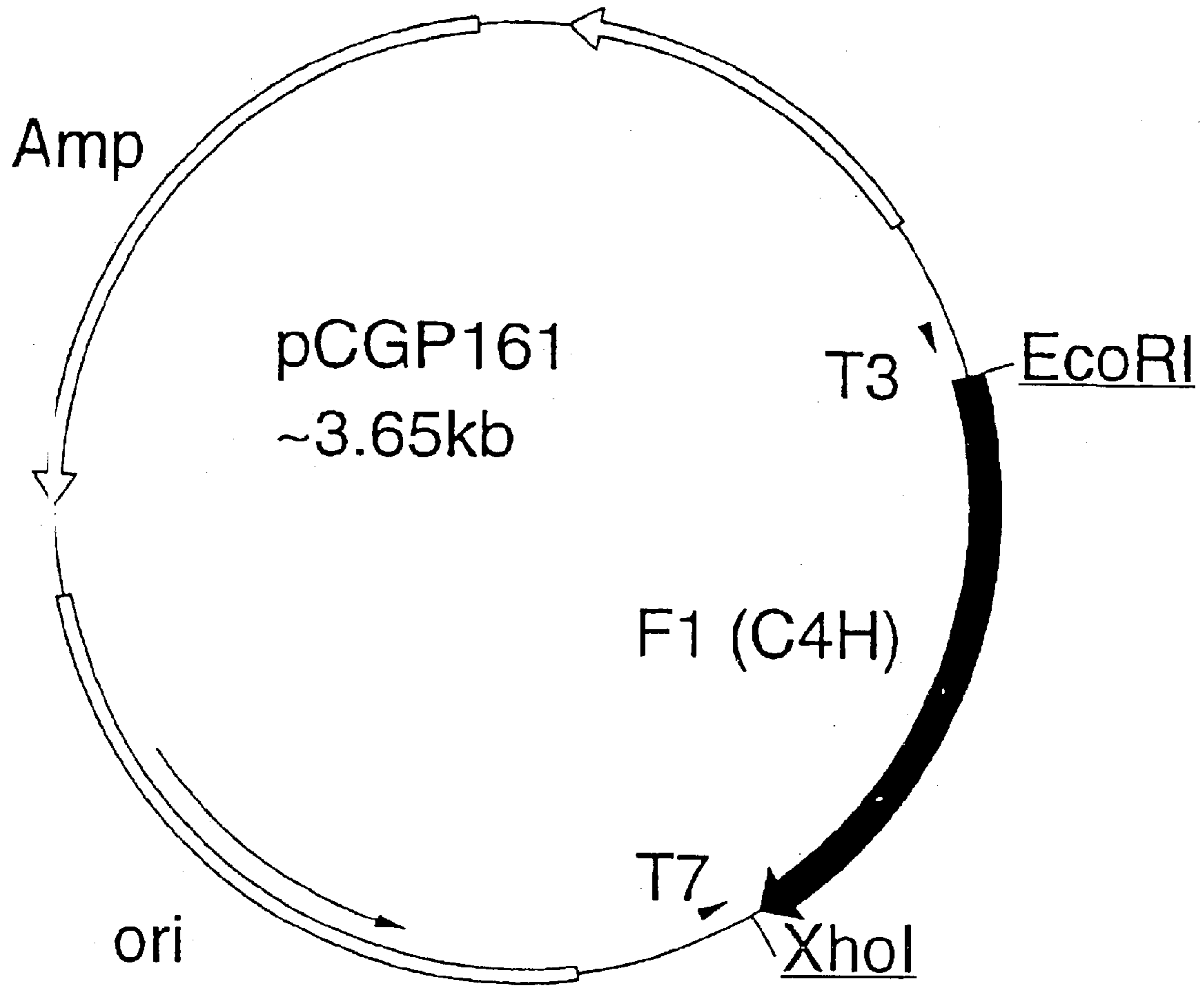


Figure 2

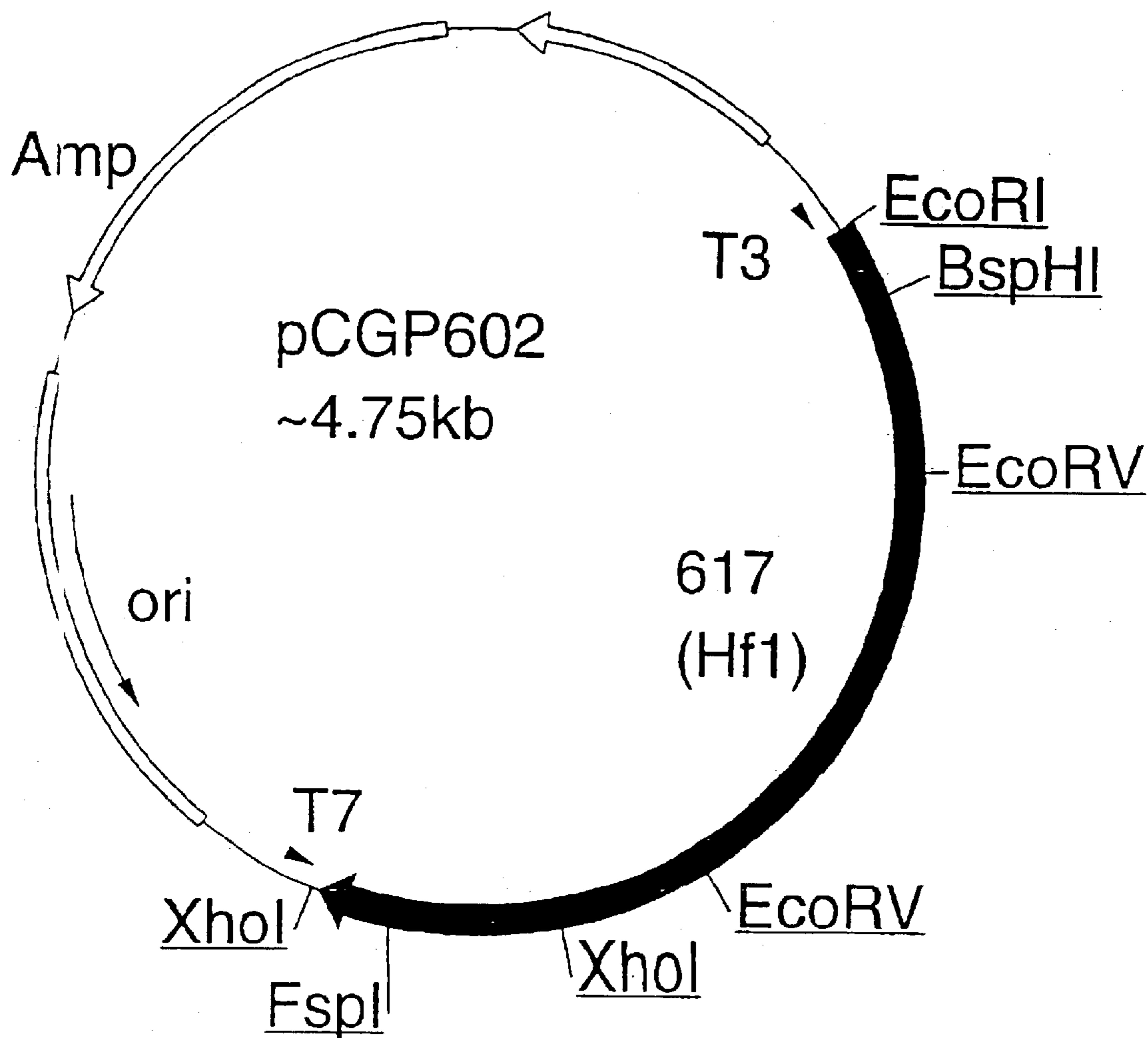


Figure 3

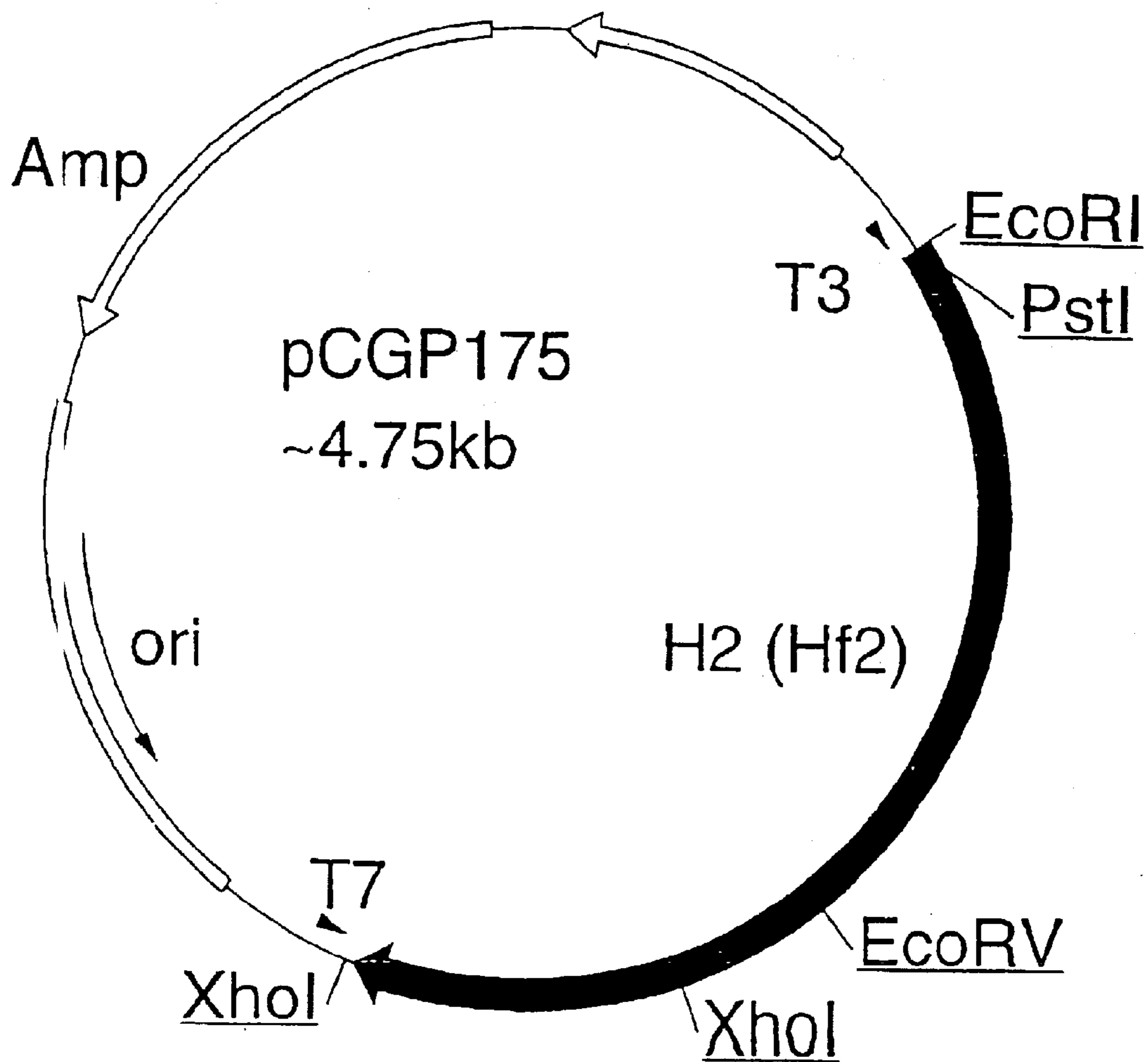


Figure 4

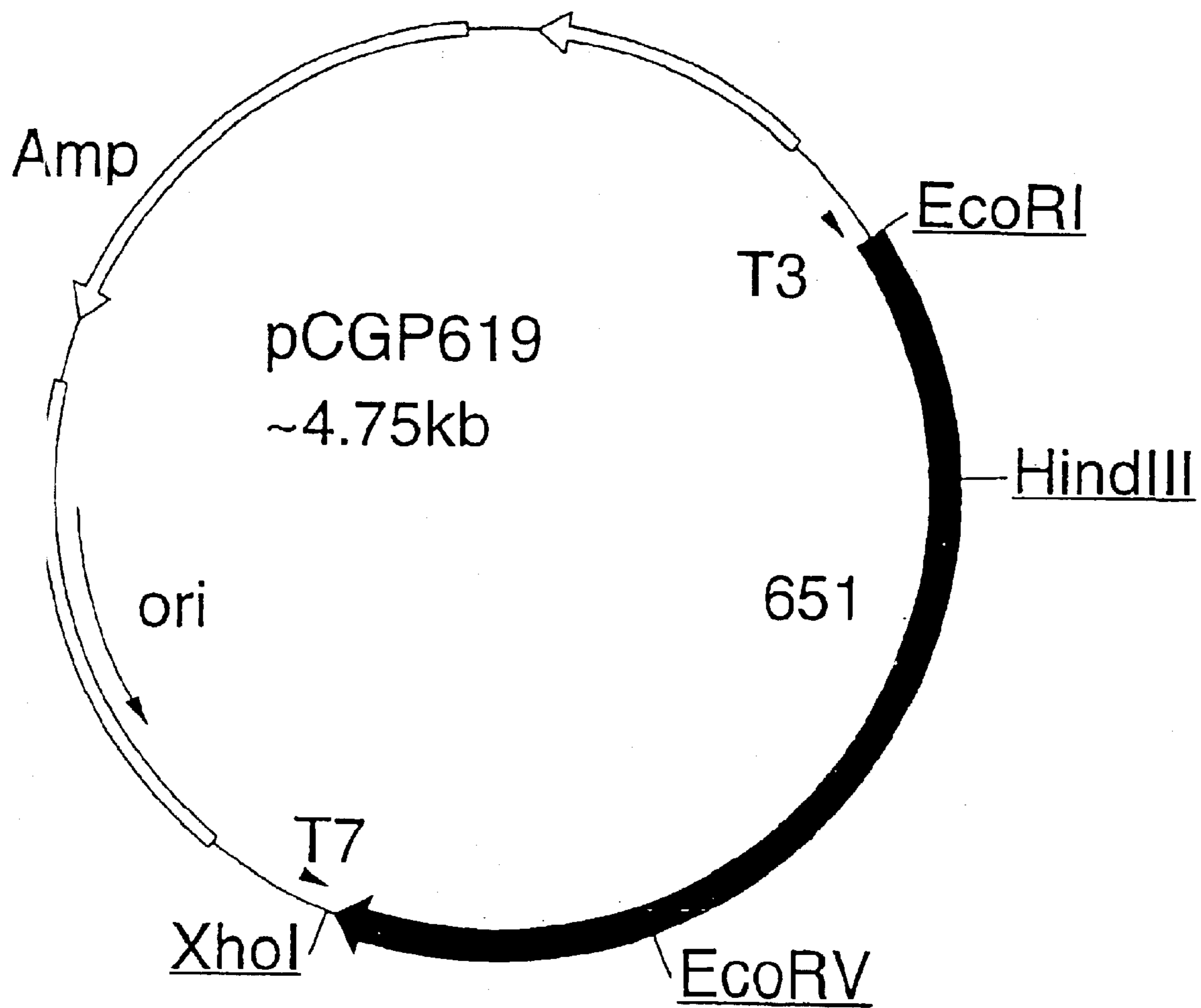


Figure 5



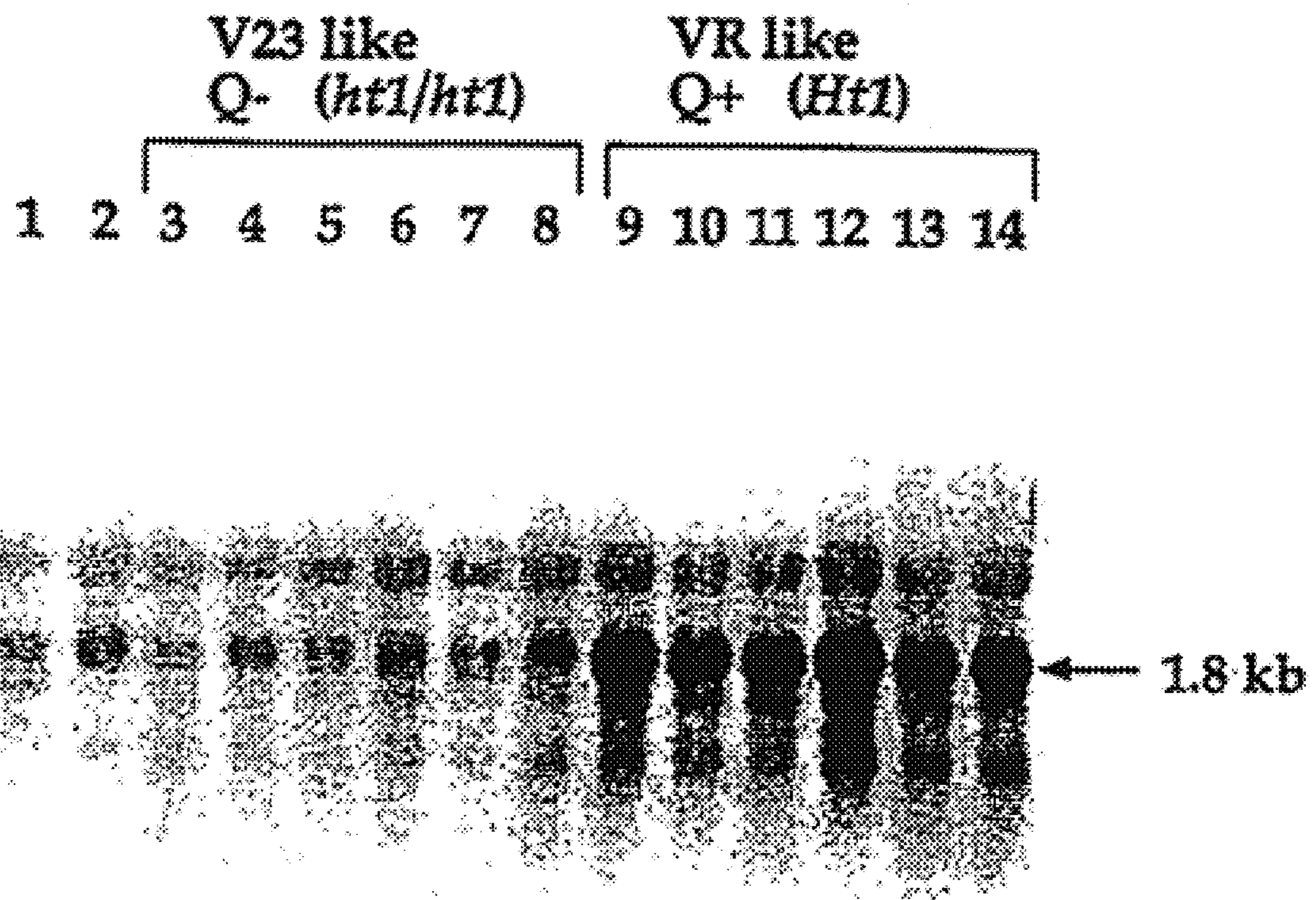


Figure 6

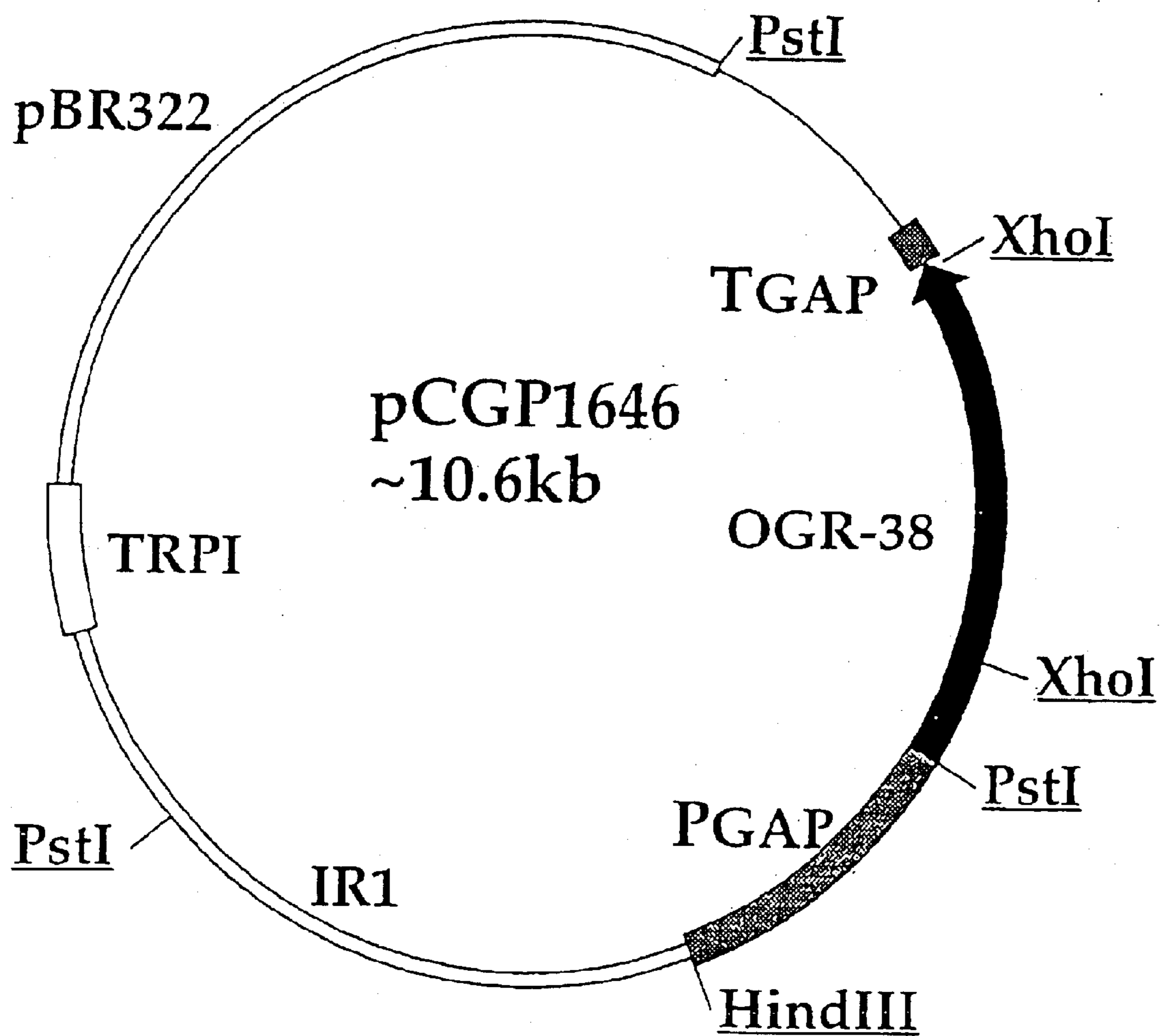


Figure 7

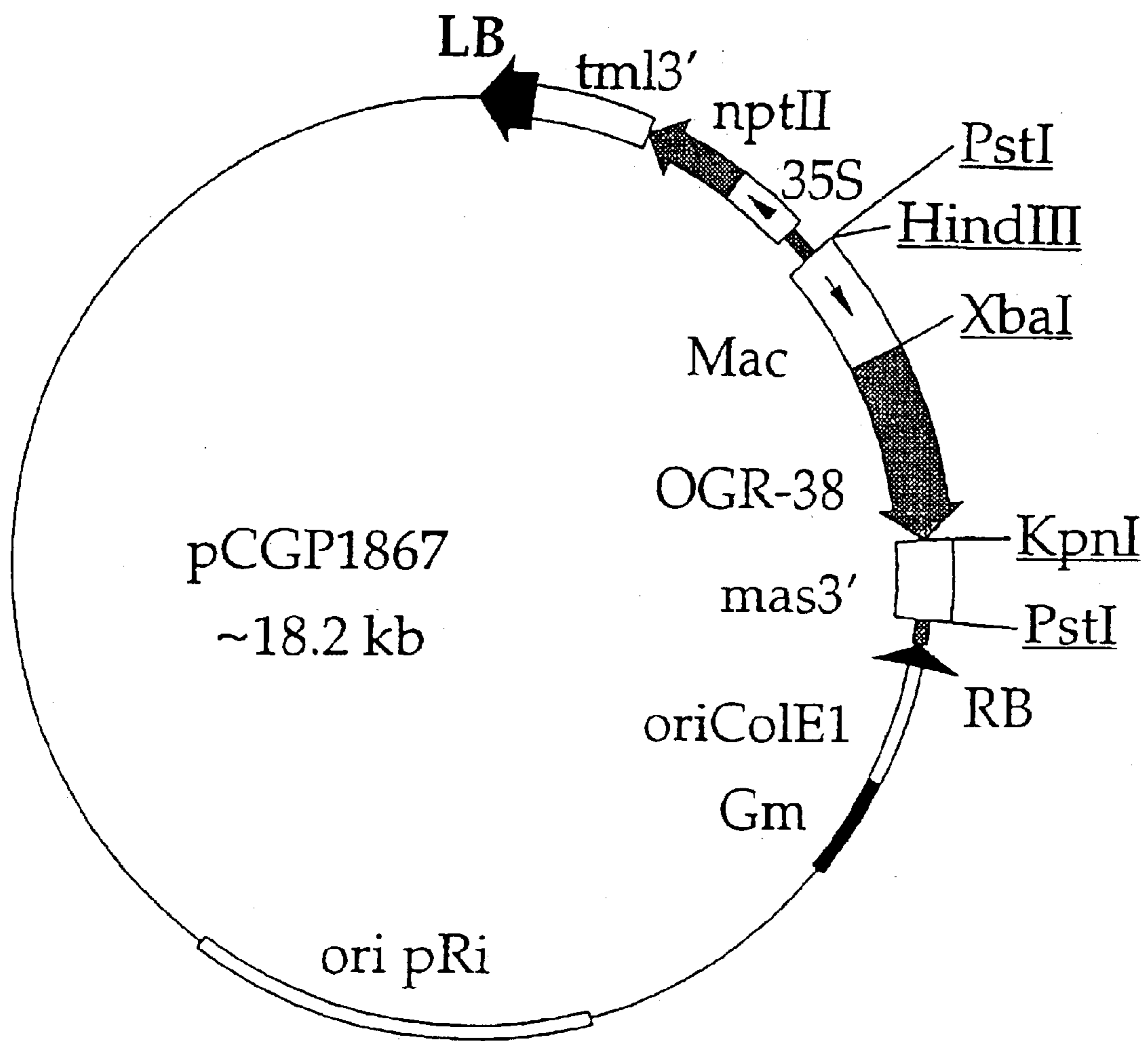


Figure 8

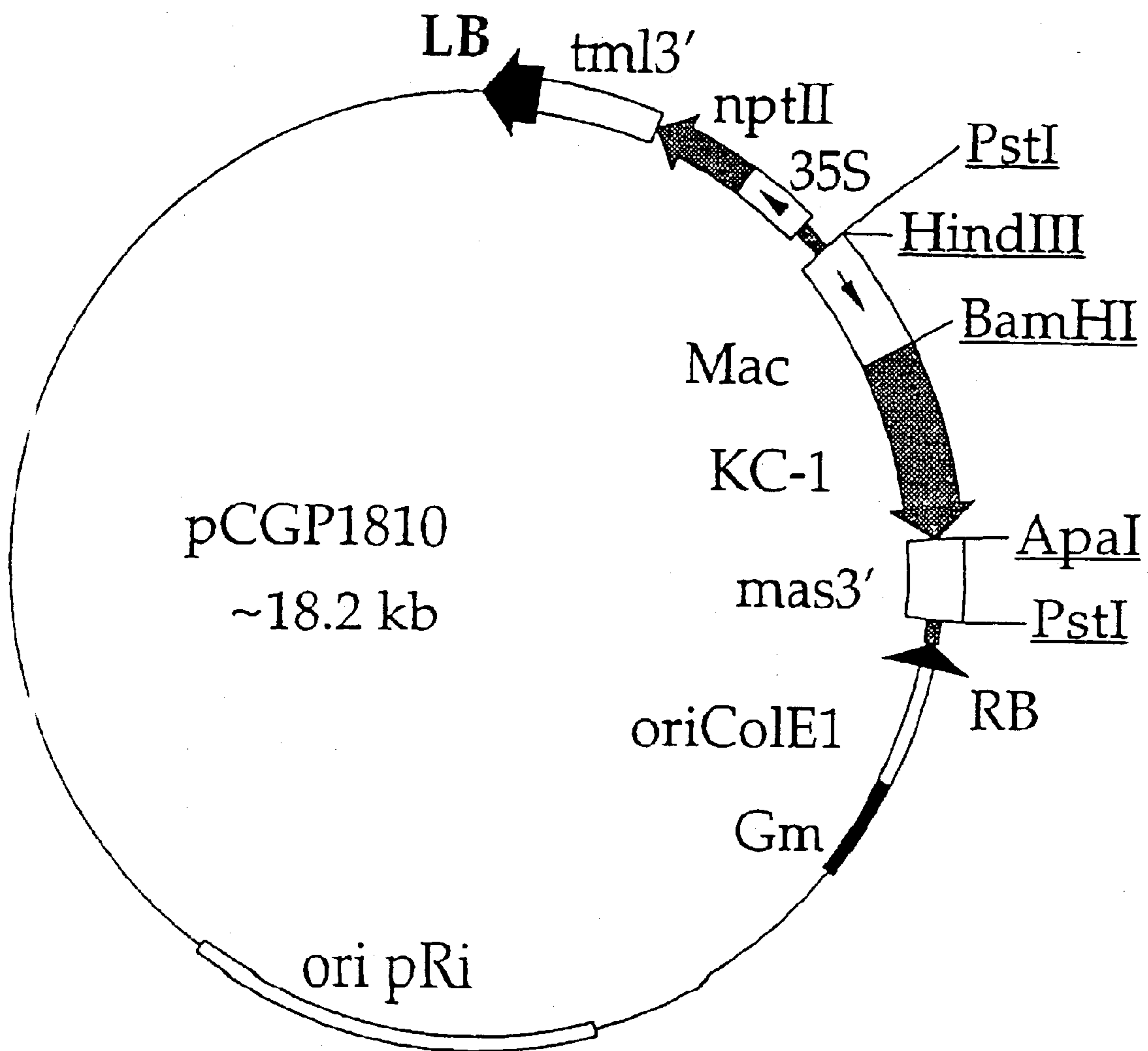


Figure 9

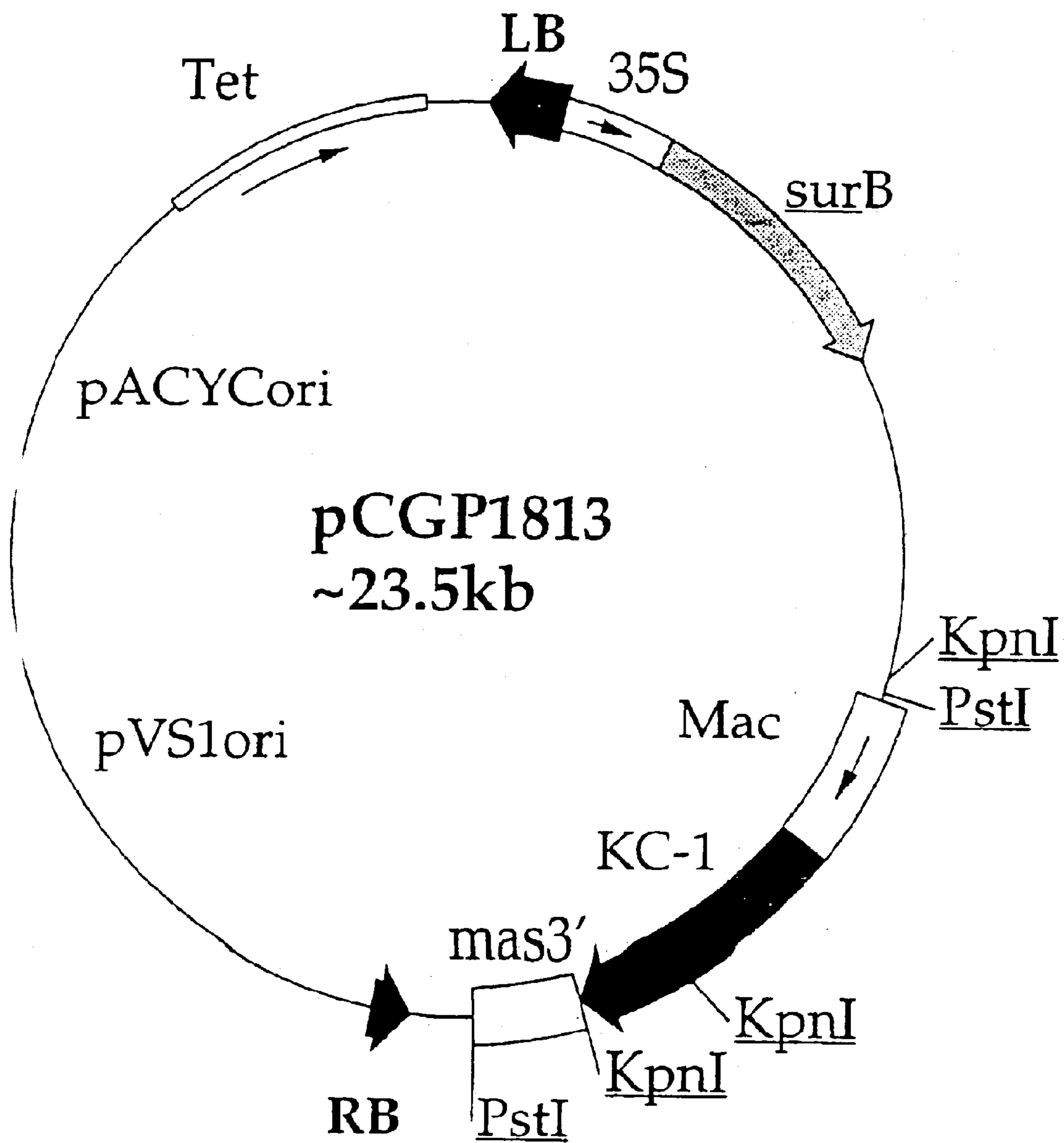


Figure 10

N8K16		N8 x K16 F2 population													
+	-	+	+	+	+	+	-	+	+	-	+	-	-	+	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	



Figure 11

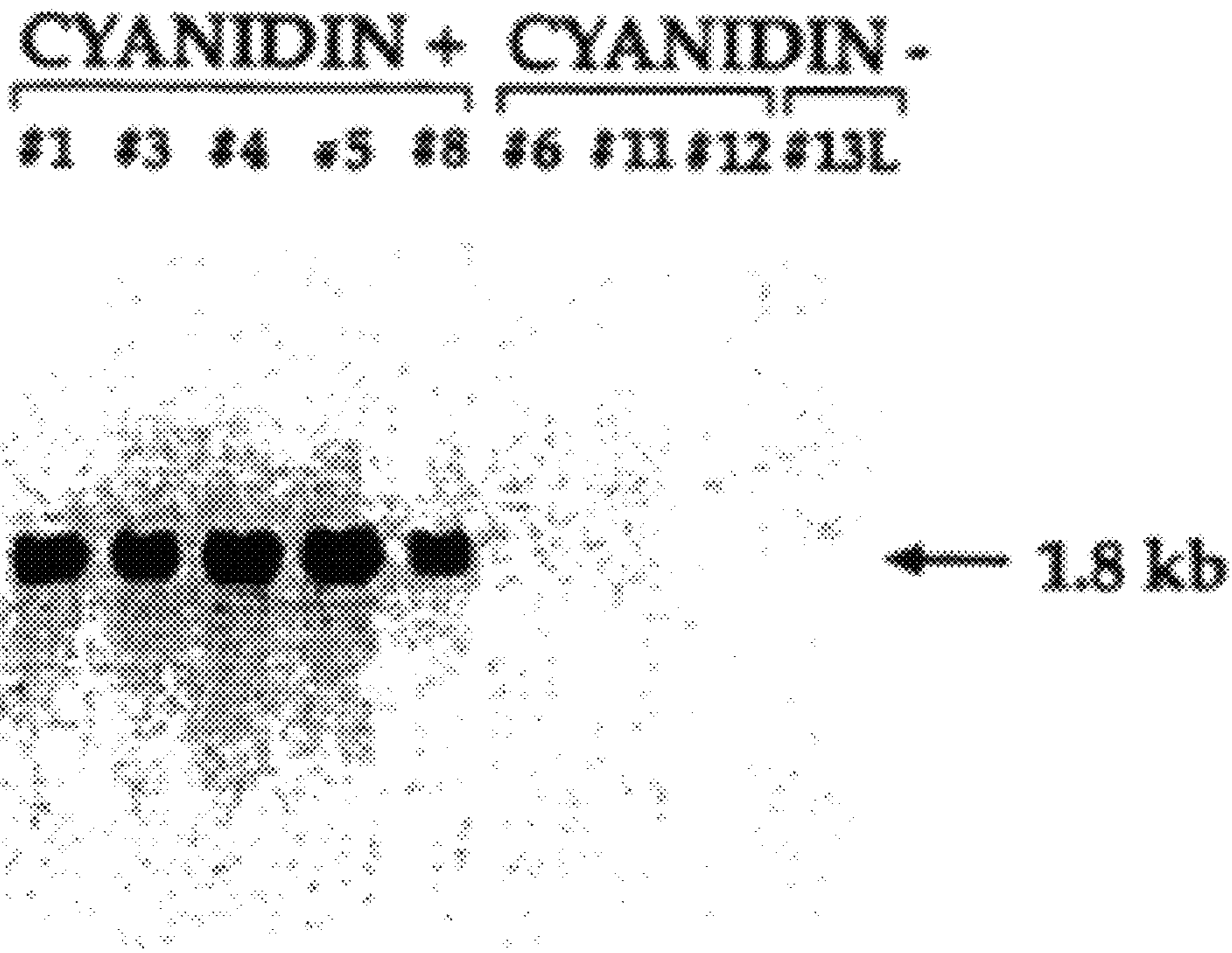


Figure 12

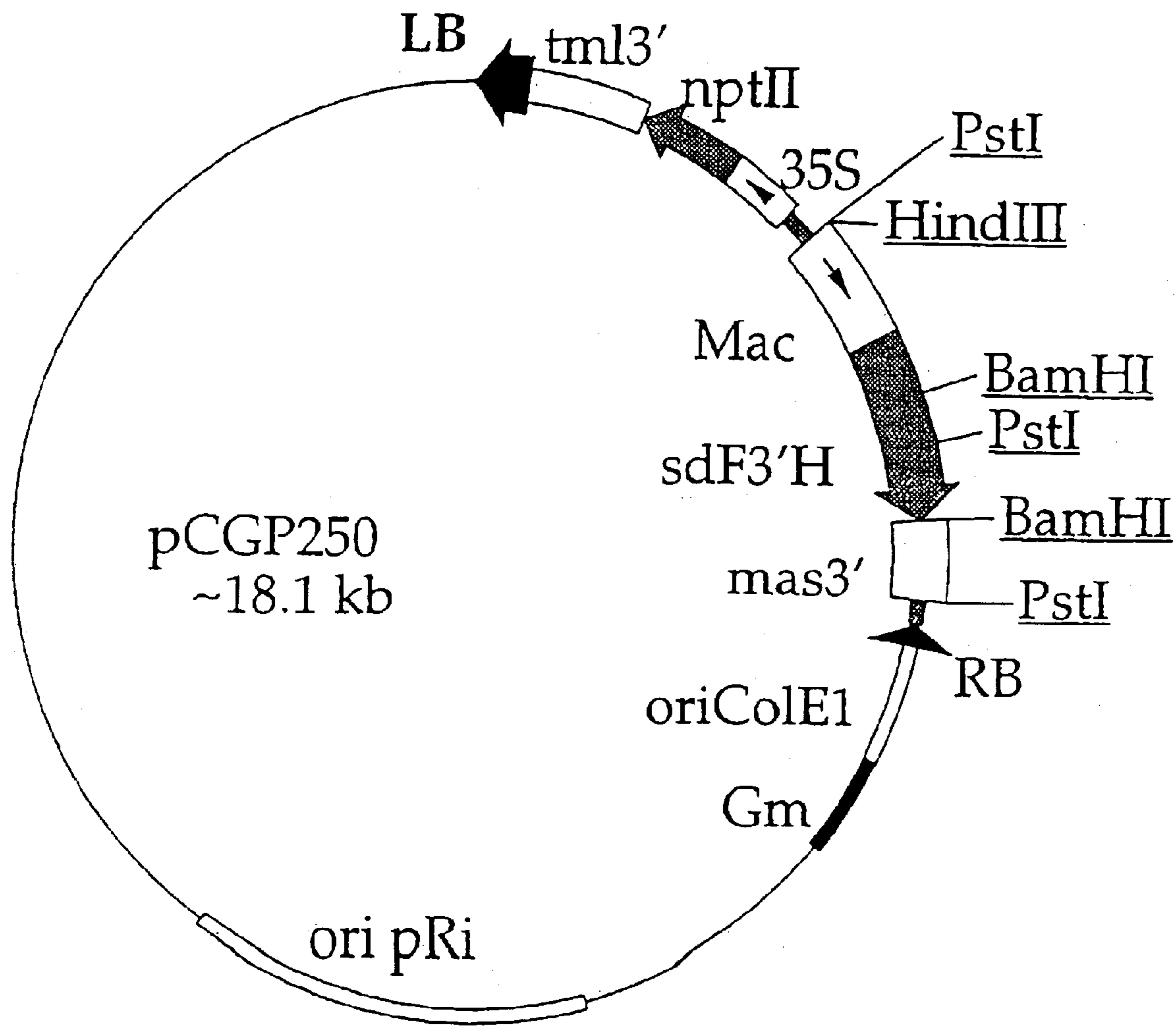


Figure 13



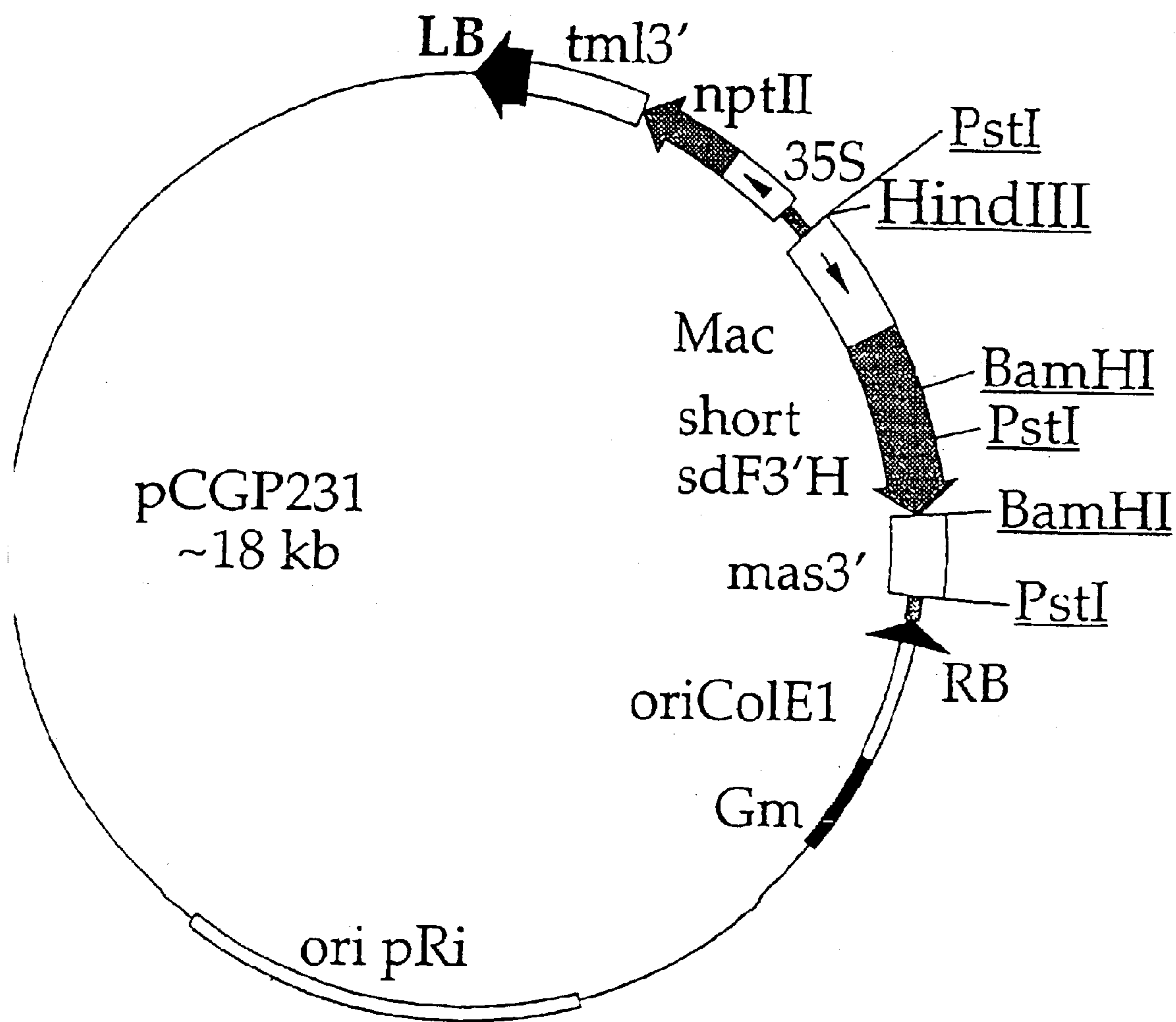


Figure 14

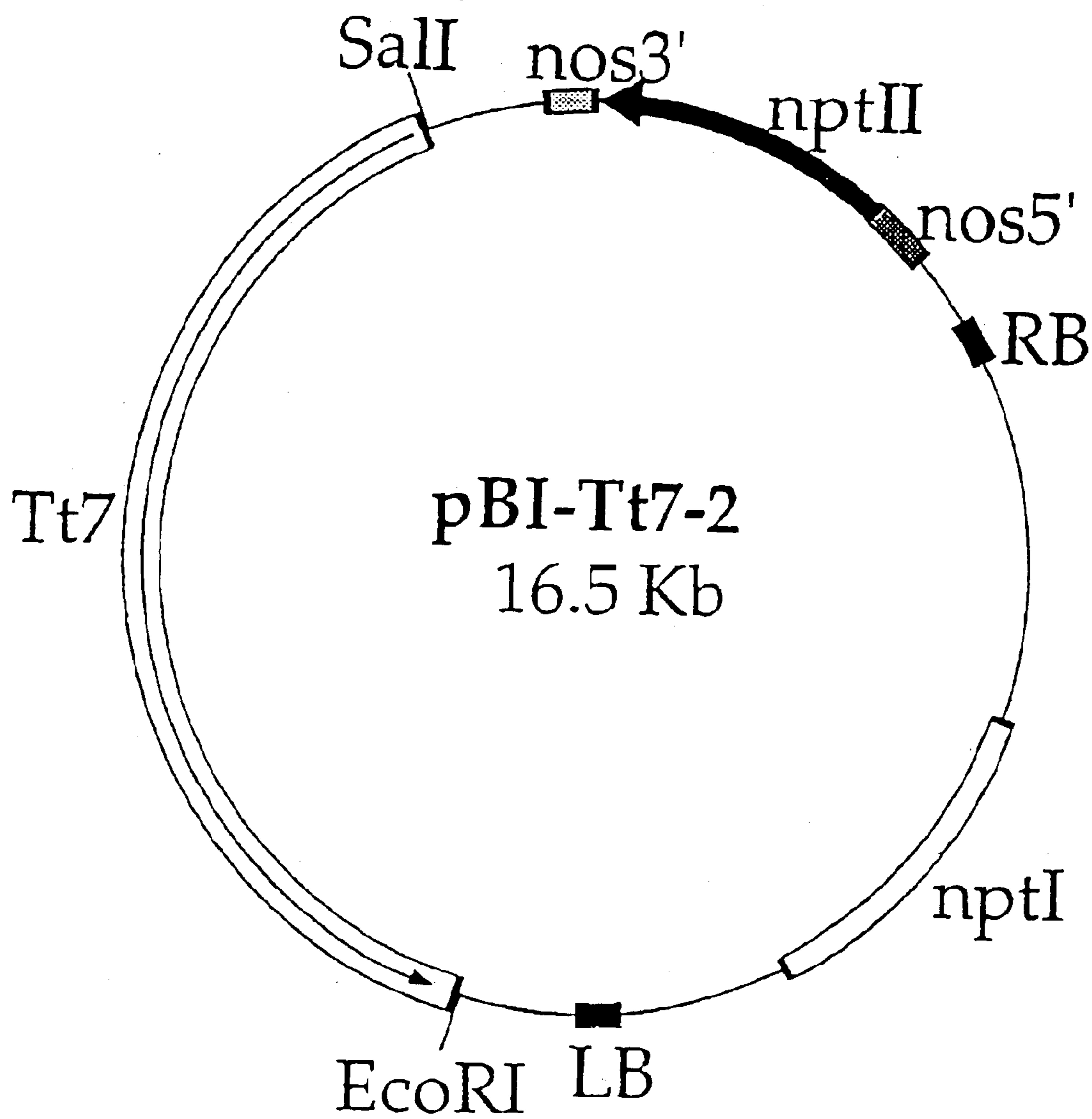


Figure 15

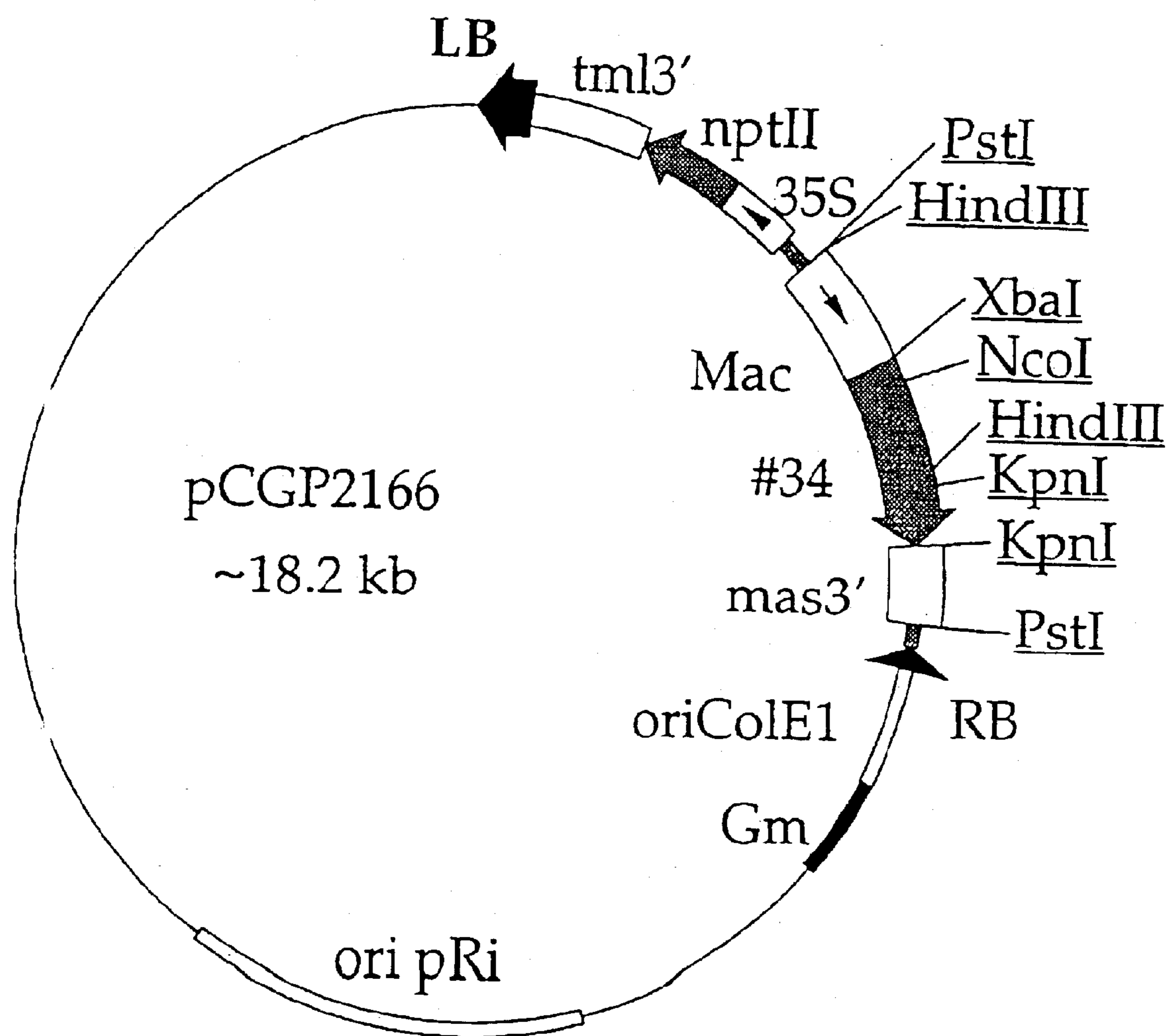


Figure 16

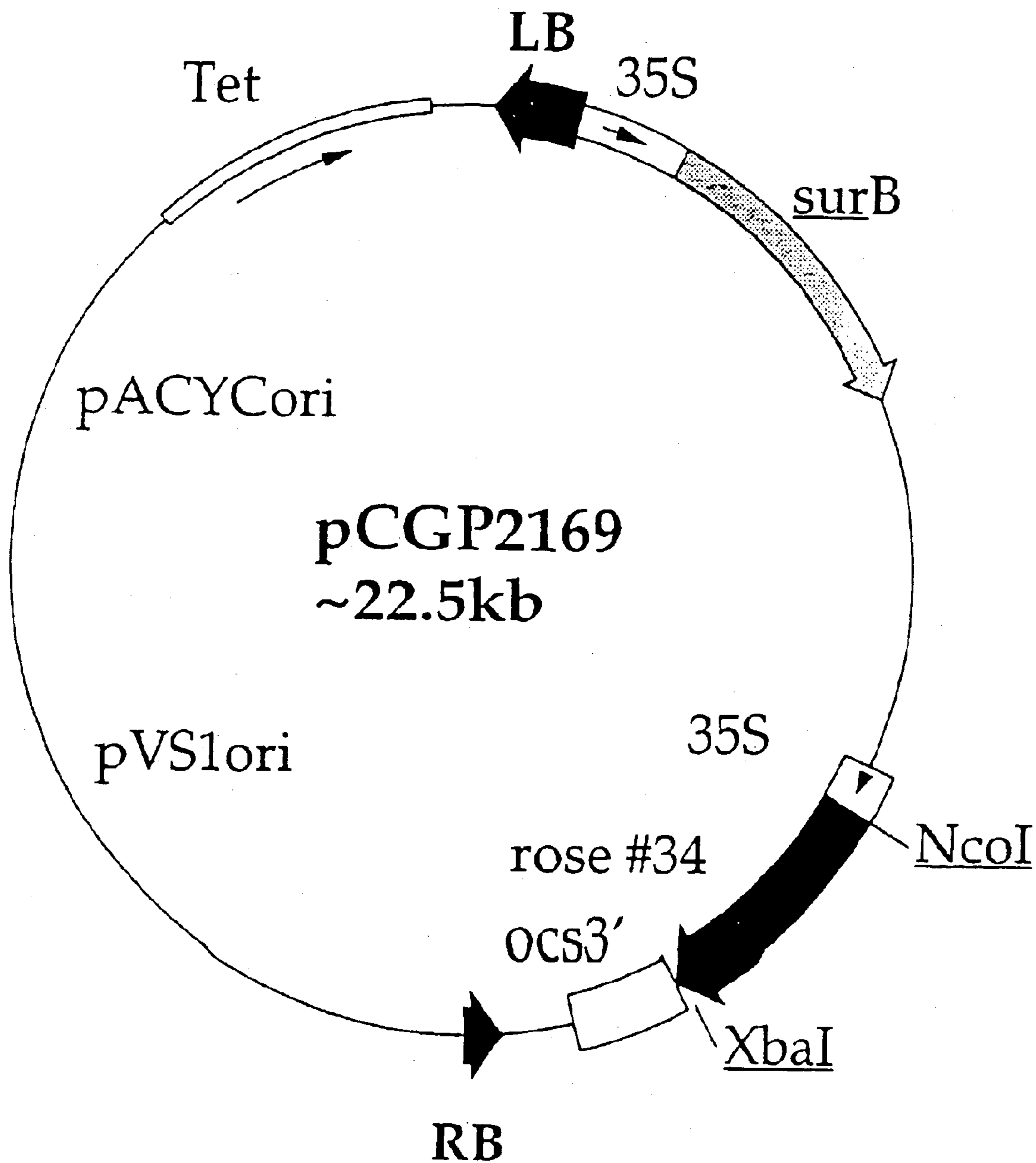


Figure 17

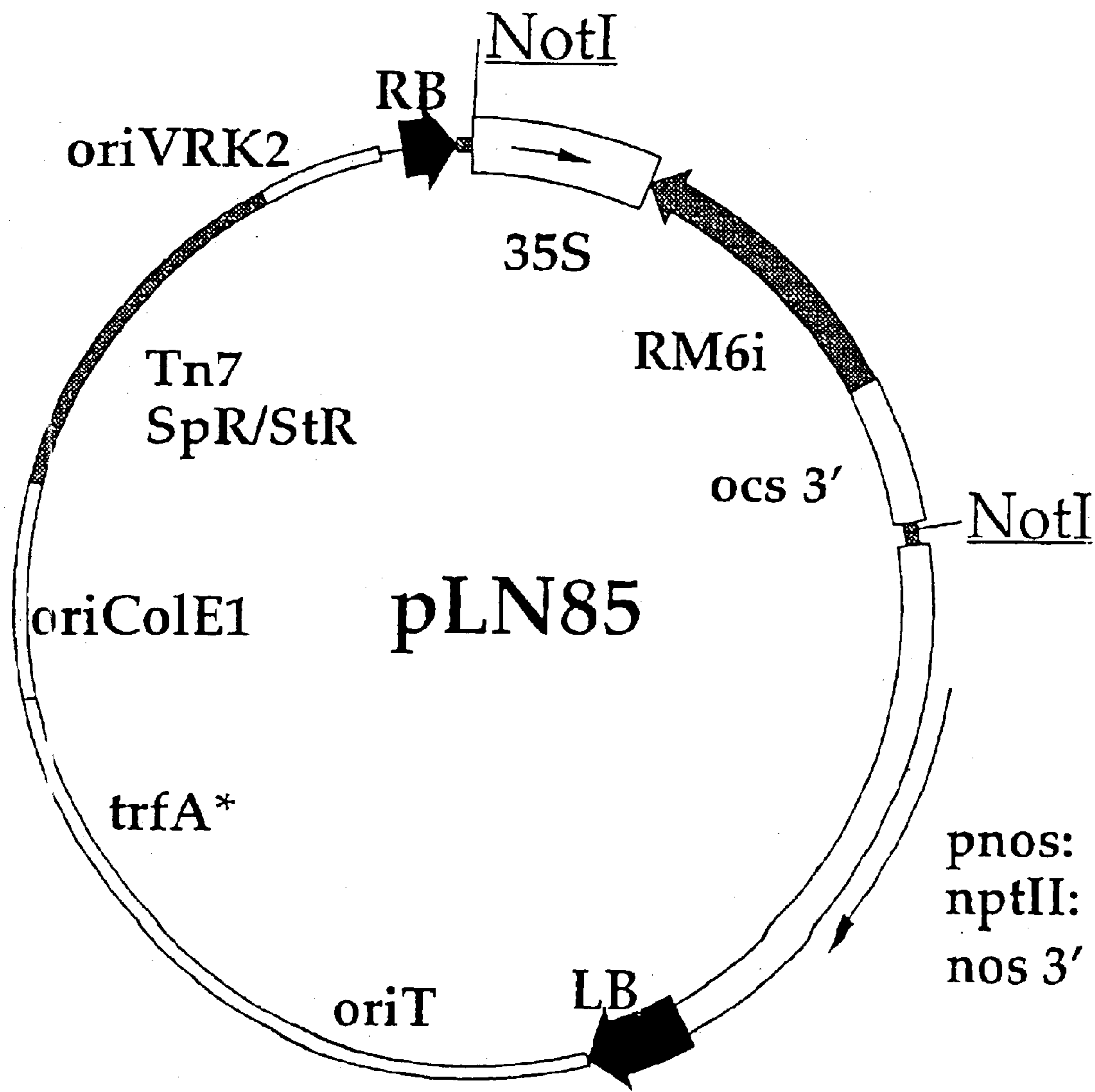


Figure 18

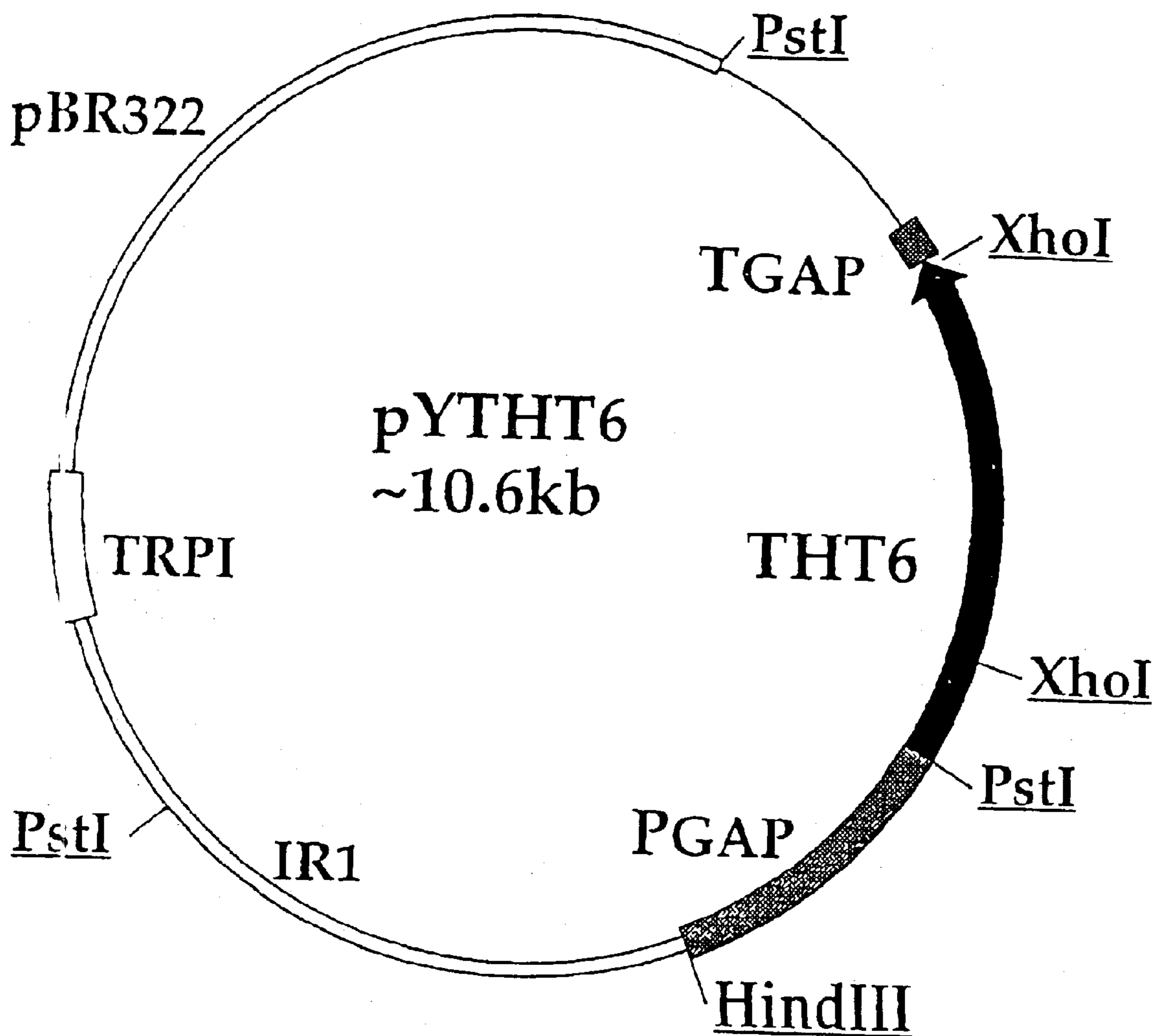


Figure 19

Figure 20 (i)

A 1 me l l s l I l y t V i f s f l L q f i L 21  
 B 1 M h n l y Y L i t t v 11  
 C 1 m q h q y y s l i t m d d i s I t s l L v p c t F I l g f L 30  
 D 1 m a t l f L t i l L a t v l F L i l r I 20  
 E 1 m f l i v v i t f l f a v F L f r l L 19  
 F 1 m t i l a f V f y a L i l g s v L y v f L 21  
 G 1 m s p l a l m i I s t l L g f l l Y h s l r L 23  
 H 1 s l t l I f c t L v f a i F L y f l I 19  
 I 1  
 J 1  
 K 1 m d y v n I l l g L f f t w F L v n g L 20

A 22 r - s f f r k R y p l p L P P G P K P W P I I G N L v H L G 50  
 B 12 f r g - - - h q k p L P P G P R P W P I V G N L P H M G 36  
 C 31 l l y s f l n K k v k p L P P G P K P W P I V G N L P H L G 60  
 D 21 f s h r r n r s h n n r L P P G P n P W P I I G N L P H M G 50  
 E 20 f s g k s q r - h s l p L P P G P K P W P V V G N L P H L G 48  
 F 22 n l s - - - s R k s a r L P P G P t P W P I V G N L P H L G 48  
 G 24 l l f s g q g R - - r l L P P G P R P W P L V G N L P H L G 51  
 H 20 l r - - v k q R y p l p L P P G P K P W P V L G N L P H L G 47  
 I 1 P I L G N I P H L G 10  
 J 1  
 K 21 m s l r - r r K i s k k L P P G P f P l P I I G N L h l L G 49

A 51 p K P H Q S t A A M A Q t Y G P L M y L K M G F V D V V V A 80  
 B 37 q a P H Q g L A A L A Q k Y G P L L y M R L G Y V D V V V A 66  
 C 61 p K P H Q S M A A L A R v h G P L I H L K M G F V h V V V A 90  
 D 51 t K P H R T L S A M v t t Y G P I L H L R L G F V D V V V A 80  
 E 49 p f P H H S I A e L A K k n G P L M H L R L G Y V D V V V A 78  
 F 49 p i P H H A L A A L A K k Y G P L M H L R L G c V D V V V A 78  
 G 52 p K P H a S M A e L A R a Y G P L M H L K M G F V h V V V A 81  
 H 48 k K P H Q S I A A M A e r Y G P L M H L R L G F V D V V V A 77  
 I 11 s K P H Q T L A e M A K t Y G P L M H L K f G l k D a V V A 40  
 J 1  
 K 50 n H P H K S L A q L A K i h G P I M N L K L G q L n t V V i 79

A 81 A S A S V A a Q P L K t H D A N F S S R P P N S G A e H M A 110  
 B 67 A S A S V A t Q P L K t H D l N F S S R P P N S G A K H I A 96  
 C 91 S S A S V A e k P L K v H D A N F S S R P P N S G A K H V A 120  
 D 81 A S k S V A e Q P L K i H D A N F A S R P P N S G A K H M A 110  
 E 79 A S A S V A a Q P L K t H D A N F S S R P P N S G A K H L A 108  
 F 79 A S A S V A a Q P L K v H D A N F A S R P P N S G A K H V A 108  
 G 82 S S A S a A e Q c L R v H D A N F l S R P P N S G A K H V A 111  
 H 78 A S A A V A a Q P L K v H D S N F S n R P P N S G A e H I A 107  
 I 41 S S A S V A e Q P L K k H D v N F S n R P P N S G A K H I A 70  
 J 1  
 K 80 S S S v V A x E v L Q k Q D l T P S n R f v p d v v H v r n 109

Figure 20 (ii)

A	111	YNYQDLVFAFYGPRWRMLRRIKCSVHLFSTK	140
B	97	YNYQDLVFAFYGPKWRMLRRIKCSLHMPSSK	126
C	121	YNYQDLVFAFYGPRWRMLRRIKCALHLFSAK	150
D	111	YNYQDLVFAFYG <b>h</b> PRWRLLRRIKSSVHLFSAK	140
E	109	YNYQDLVFAFY <b>r</b> PGPRWRM <b>f</b> RRIKSSVHLF <b>g</b> SK	138
F	109	YNYQDLVFAFYGPRWRLLRRIKCSVHLFSAK	138
G	112	YNYEDLVFAFYGPKWRLLRRIKCA <b>q</b> HIF <b>v</b> SK	141
H	108	YNYQDLVFAFYGPRWRMLRRIKTBVHLFSAK	137
I	71	YNYQDLVFAFYGPRWRLLRRIKCSVHLFSSK	100
J	1		0
K	110	h s d f s <b>V V</b> w l <b>P v</b> <b>N s</b> <b>R W K t</b> <b>L R K I</b> m n s s <b>I P S</b> g n	139

A	141	A L D D F R H V R Q D - - - E V <b>k t</b> L T R A L A s A G q k P	167
B	127	A L D D F R <b>l</b> V R Q E - - - E V S I L <b>v n</b> A I A k A G t k P	153
C	151	A L <b>n</b> D F <b>t</b> H V R Q D - - - E V <b>g i</b> L T R <b>v l</b> A d A G e t P	177
D	141	A L E D F K H V R Q E - - - E V <b>g t</b> L T R e <b>l v r v</b> G t k P	167
E	139	A L D D <b>l</b> K H V R Q E - - - E V S V L A H A L A n S G s k v	165
F	139	A L D D F R H V R Q E - - - E V A V L T R <b>v l</b> l s A G n s P	165
G	142	A M D D F R R V R E E - - - E V A I L S R A L A - - G k r a	166
H	138	A L D D F <b>c</b> H V R Q E - - - E V A <b>t</b> L T R S L A s A G k t P	164
I	101	A L D D F Q H V R <b>h</b> E - - - E I C I L <b>l</b> R A I A s g G h a P	127
J	1		14
K	140	k <b>L D</b> g n <b>Q H L R</b> s k k v q <b>E L</b> i d y <b>C Q</b> k c A k n G e - a	168

A	168	V <b>k</b> L G Q L L N V C T T N A L A R V M L G K R V F a d <b>G s G</b>	197
B	154	V Q L G Q L L N V C T T N A L S R V M L G K R V <b>l G</b> d <b>G t G</b>	183
C	178	L <b>k</b> L G Q M M N <b>c</b> C A T N A I A R V M L G R R V <b>v G</b> h a d -	206
D	168	V N L G Q L V N M C <b>v v</b> N A L <b>g r</b> e M I G R R L F G - - - a	194
E	166	V N L <b>a</b> Q L L N L C T V N A L <b>g r</b> V M V G R R V F G d <b>G S G</b>	195
F	166	V Q L G Q L L N V C A T N A L A R V M L G R R V F G - - - d	192
G	167	V <b>p</b> I G Q M L N V C A T N A L S R V M M G R R V <b>v G</b> h a d <b>G</b>	196
H	165	V <b>k</b> L G Q L L N V C T T N A L A R V M L G R K V F N d <b>G g s</b>	194
I	128	V N L G K L L G V C T T N A L A R V M L G R R V F e - <b>G d G</b>	156
J	15	I N L G Q L L G V C T T N A L A R V M L G R R V F G d <b>G s G</b>	4
K	169	V <b>d</b> I G R a t f g T T I N I L S n t I f s K d L t N - - -	194

A	198	d v D P Q A <b>a</b> E F K S M V V E M M V V A G V F N I G D F I P	227
B	184	k s D P K A E E F K <b>d</b> M V L E L M V L T G V F N I G D F V P	213
C	207	- - - s K A E E F K A M V V E L M V L A G V F N L G D F I P	233
D	195	d a <b>D h</b> K A D E F R S M V <b>t</b> E M M <b>a</b> L A G V F N I G D F V P	224
E	196	g d D P K A D E F K S M V V E M M V L A G V F N I G D F I P	225
F	193	g i <b>D r</b> s <b>a</b> n E F K <b>d</b> M V V E L M V L A G e F N L G D F I P	222
G	197	t n <b>D a</b> K A E E F K A M V V E L M V L S G V F N I G D F I P	226
H	195	k s D P K A E E F K S M V <b>e</b> E M M V L A G s F N I G D F I P	224
I	157	g e n P H A D E F K S M V V E I M V L A G a F N L G D F I P	186
J	45	g v D P Q A D E F K S M V V E I M V L A G a F N L G D F I P	74
K	195	p f s d s <b>A k</b> E F K e L V w n I M V e A G k p N L v D Y f P	224





Figure 20 (iv)

A	341	d	R L V g E l D L a	Q L t Y L E A I V K E T F R L H P S T P	370
B	327	d	R L I A E A D I p	N L t Y f Q A V I K E v F R L H P S T P	356
C	349	n	R V V k E A D L a	g L P P L Q A V V K E N F R L H P S T P	378
D	339	d	R p V n E S D I a	Q L P Y L Q A V I K E N F R L H P p T P	368
E	339	d	R L V T E S D I p	N L P Y L Q A V I K E T F R L H P S T P	368
F	336	d	R L V T E l D L s	Q L t Y L Q A L V K E v F R L H P S T P	365
G	339	n	R L V S E T D L n	Q L P Y L Q A V V K E T F R L H P p T P	368
H	345	n	Q L V T E S D L t	d L P F L Q A I V K E T F R L H P S T P	374
I	305	n	R f V T E S D L p	Q L t F L Q A V I K E T F R L H P S T P	334
J	192	s	R L V T E S D L p	Q L a P L Q A V I K E T F R L H P S T P	221
K	337	g	K q V e E A D V g	r L P Y L r C I V K E T l R I H P A A P	366

A	371	L S L P R I A S E S C E I N G Y f	I P K G S T L L L N V W A	400
B	357	L S L P R V A n E S C E I N G Y h	I P K N T T L L V N V W A	386
C	379	L S L P R I A h E S C E V N G Y l	I P K G S T L L L N V W A	408
D	369	L S L P H I A S E S C E I N G Y h	I P K G S T L L t N I W A	398
E	369	L S L P R M A A E S C E I N G Y h	I P K G S T L L V N V W A	398
F	366	L S L P R I S S E S C E V d G Y y	I P K G S T L L V N V W A	395
G	369	L S L P R L A e d d C E I d G Y l	I P K G S T L L V N V W A	398
H	375	L S L P R M g A Q g C E I N G Y f	I P K G A T L L V N V W A	404
I	335	L S L P R M A A E d C E I N G Y y	V s e G S T L L V N V W A	364
J	222	L S L P R M A S E g C E I N G Y s	I P K G S T L L V N V W S	251
K	367	L l I P R k v e E d v E L s t Y i	I P K d S q V L V N V W A	396

A	401	I A R D P n a	W A D P L E F R P E R F L P G G E K P k V D V	430
B	387	I A R D P e	V W A D P L E F K P E R F L P G G E K P N V D V	416
C	409	I A R D P n	V W d E F L E F R P E R F L k G G E K P N V D V	438
D	399	I A R D P d	q W S D P L a F K P E R F L P G G E K s G V D V	428
E	399	I S R D P a	e W A D P L E F K P E R F L P G G E K P N V D I	428
F	396	I A R D P k	M W A D P L E F R P s R F L P G G E K P G a D V	425
G	399	I A R D P k	V W A D P L E F R P E R F L t G G E K a d V D V	428
H	405	I A R D P n	V W T n P L E F n P h R F L P G G E K P N V D I	434
I	365	I A R D P n	a W A n P L D P n P t R F L a G G E K P N V D V	394
J	252	I A R D P s	I W A D P L E F R P a R F L P G G E K P N V D V	281
K	397	I g R n s d	L W e n P L V F K P E R F w e s - - - e I D I	422

A	431	R G N D F E V I P F G A G R R I C A G M n	L G I R M V Q L M	460
B	417	K G N D F E L I P F G A G R R I C A G L S L	G L R M V Q L M	446
C	439	R G N D F E L I P F G A G R R I C A G M S L	G I R M V Q L L	468
D	429	K G s D F E L I P F G A G R R I C A G L S L	G L R t I Q f L	458
E	429	R G N D F E V I P F G A G R R I C A G M S L	G L R M V H L M	458
F	426	R G N D F E V I P F G A G R R I C A G M S L	G L R M V Q L L	455
G	429	K G N D F E V I P F G A G R R I C A G V g	L G I R M V Q L L	458
H	435	K G N D F E V I P F G A G R R I C S G M S L	G I R M V H L L	464
I	395	K G N D F E V I P F G A G R R I C A G M S L	G I R M V Q L V	424
J	282	R G N D F E V I P F G A G R R I C A G M S L	G L R M V Q L s	311
K	423	R G r D F E L I P F G A G R R I C p G L p L	a M R M I p V a	452

Figure 20 (v)

A	461	i	A	T	L	I	H	A	F	n	W	D	L	v	s	G	q	l	P	E	m	L	N	M	E	E	A	Y	G	L	T	490
B	447	T	A	T	L	a	H	T	Y	D	W	a	L	A	d	G	L	m	P	E	k	L	N	M	D	E	A	Y	G	L	T	476
C	469	T	A	T	L	n	H	A	F	D	f	D	L	A	d	G	q	l	P	E	s	L	N	M	E	E	A	Y	G	L	T	498
D	459	T	A	T	L	V	Q	g	F	D	W	E	L	A	G	G	V	t	P	E	k	L	N	M	E	E	S	Y	G	L	T	488
E	459	T	A	T	L	V	H	A	F	n	W	a	L	A	d	G	L	t	a	E	k	L	N	M	D	E	A	Y	G	L	T	488
F	456	i	A	T	L	V	Q	T	F	D	W	E	L	A	N	G	L	e	P	E	m	L	N	M	E	E	A	Y	G	L	T	485
G	459	T	A	S	L	I	H	A	F	D	l	D	L	A	N	G	L	l	a	Q	n	L	N	M	E	E	A	Y	G	L	T	488
H	465	v	A	T	L	V	H	A	F	D	W	D	L	v	N	Q	q	s	v	E	t	L	N	M	E	E	A	Y	G	L	T	494
I	425	T	A	S	L	V	E	S	F	D	W	a	L	l	d	G	L	k	P	E	k	L	d	M	E	E	g	Y	G	L	T	454
J	312	T	A	T	L	V	H	S	F	n	W	D	L	l	N	O	M	s	p	d	k	L	d	M	E	E	A	Y	G	L	T	341
K	453	l	g	S	L	L	n	S	F	n	W	k	L	y	G	G	I	a	P	k	d	L	d	M	q	E	k	F	G	I	T	482

A	491	L	Q	R	A	d	P	L	V	V	H	P	R	P	R	L	e	a	Q	a	Y	i	g	512			
B	477	L	Q	R	k	v	P	L	M	V	H	P	t	r	R	L	S	a	R	V	Y	n	s	g	f	500	
C	499	L	Q	R	A	d	P	L	V	V	H	P	K	P	R	512											
D	489	L	Q	R	A	v	P	L	V	V	H	P	K	P	R	L	A	p	n	V	Y	g	l	g	s	g	513
E	489	L	Q	R	A	a	P	L	M	V	H	P	R	t	R	L	A	p	Q	a	Y	k	t	s	s	512	
F	486	L	Q	R	A	a	P	L	M	V	H	P	K	P	R	L	A	p	H	V	Y	e	s	i	508		
G	489	L	Q	R	A	e	P	L	L	V	H	P	R	P	R	L	A	t	H	V	Y	508					
H	495	L	Q	R	A	v	P	L	M	L	H	P	K	P	R	L	q	p	H	L	Y	t	l	n	517		
I	455	L	Q	R	A	s	P	L	I	V	H	P	K	P	R	L	S	a	Q	V	Y	c	m	476			
J	342	L	Q	R	A	s	P	L	I	V	H	P	K	P	R	L	A	s	s	M	Y	v	k	363			
K	483	L	a	K	A	q	P	L	L	a	i	P	t	P	l	496											

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**NUCLEIC ACID SEQUENCES ENCODING  
FLAVONOID 3'-HYDROXYLASE AND  
METHODS OF ALTERING FLOWER COLOR  
THEREWITH**

FIELD OF THE INVENTION

The present invention relates generally to genetic sequences encoding flavonoid pathway metabolising enzymes and more particularly to flavonoid 3'-hydroxylase (hereinafter referred to as "F3'H") or derivatives thereof and their use in the manipulation of pigmentation in plants and other organisms.

Bibliographic details of the publications referred to by the author in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs) for the nucleotide and amino acid sequences referred to in the specification and claims are defined following the bibliography. A summary of the SEQ ID NOs, and the sequences to which they relate, is provided prior to the Examples.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

DESCRIPTION OF THE RELATED ART

The rapidly developing sophistication of recombinant DNA technology is greatly facilitating research and development in a range of biotechnology related industries. The horticultural industry has become a recent beneficiary of this technology which has contributed to developments in disease resistance in plants and flowers exhibiting delayed senescence after cutting. Some attention has also been directed to manipulating flower colour.

The flower industry strives to develop new and different varieties of flowering plants. An effective way to create such novel varieties is through the manipulation of flower colour. Classical breeding techniques have been used with some success to produce a wide range of colours for most of the commercial varieties of flowers. This approach has been limited, however, by the constraints of a particular species' gene pool and for this reason it is rare for a single species to have a full spectrum of coloured varieties. In addition, traditional breeding techniques lack precision. The aesthetic appeal of the flower is a combination of many factors such as form, scent and colour; modification of one character through hybridization can often be at the expense of an equally valuable feature. The ability to genetically engineer precise colour changes in cutflower and ornamental species would offer significant commercial opportunities in an industry which has rapid product turnover and: where novelty is an important market characteristic.

Flower colour is predominantly due to two types of pigment: flavonoids and carotenoids. Flavonoids contribute to a range of colours from yellow to red to blue. Carotenoids impart an orange or yellow tinge and are commonly the major pigment in yellow or orange flowers. The flavonoid molecules which make the major contribution to flower colour are the anthocyanins which are glycosylated derivatives of cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin, and are localised in the vacuole. The different anthocyanins can produce marked differences in colour. Flower colour is also influenced by co-pigmentation with colourless flavonoids, metal complexation, glycosylation, acylation and vacuolar pH (Forkmann, 1991).

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The biosynthetic pathway for the flavonoid pigments (hereinafter referred to as the "flavonoid pathway") is well established and is shown in FIGS. 1a and 1b (Ebel and Hahlbrock, 1988; Hahlbrock and Grisebach, 1979; Wiering and De Vlaming, 1984; Schram et al., 1984; Stafford, 1990; Van Tunen and Mol, 1990; Dooner et al, 1991; Martin and Gerats, 1993; Holton and Cornish, 1995). The first committed step in the pathway involves the condensation of three molecules of malonyl-CoA with one molecule of peoumaroyl-CoA. This reaction is catalysed by the enzyme chalcone synthase (CHS). The product of this reaction, 2',4,4',6', tetrahydroxy-chalcone, is normally rapidly isomerized to produce naringenin by the enzyme chalcone flavanone isomerase (CHI). Naringenin is subsequently hydroxylated at the 3 position of the central ring by flavanone 3-hydroxylase (F3H) to produce dihydrokaempferol (DHK).

The pattern of hydroxylation of the B-ring of DHK plays a key role in determining petal colour. The B-ring can be hydroxylated at either the 3', or both the 3' and 5' positions, to produce dihydroquercetin (DHQ) and dihydromyricetin (DHM), respectively. Two key enzymes involved in this pathway are flavonoid 3'-hydroxylase and flavonoid 3',5'-hydroxylase, both of the cytochrome P450 class. Cytochrome P450 enzymes are widespread in nature and genes have been isolated and sequenced from vertebrates, insects, yeasts, fungi, bacteria and plants.

Flavonoid 3'-hydroxylase acts on DHK to produce DHQ and on naringenin to produce eriodictyol. Reduction and glycosylation of DHQ produces the cyanidin-glycoside and peonidin-glycoside pigments which, in many plant species (for example rose, carnation and chrysanthemum), contribute to red and pink flower colour. The synthesis of these anthocyanins can also result in other flower colours. For example, blue cornflowers contain emcyan. The ability to control flavonoid 3'-hydroxylase activity, or other enzymes involved in the flavonoid pathway, in flowering plants would provide a means to manipulate petal colour. Different coloured versions of a single cultivar could thereby be generated and in some instances a single species would be able to produce a broader spectrum of colours.

A nucleotide sequence (referred to herein as SEQ ID NO:26) encoding a petunia flavonoid 3'-hydroxylase has been cloned (see International Patent Application No. PCT/AU93/00127 [WO 93/120206]). However, this sequence was inefficient in its ability to modulate the production of 3'-hydroxylated anthocyanins in plants. There is a need, therefore, to identify further genetic sequences encoding flavonoid 3'-hydroxylases which efficiently modulate the hydroxylation of flavonoid compounds in plants. More particularly, there is a need to identify further genetic sequences encoding flavonoid 3'-hydroxylases which efficiently modulate the production of 3'-hydroxylated anthocyanins in plants.

SUMMARY OF THE INVENTION

In accordance with the present invention, genetic sequences encoding flavonoid 3'-hydroxylase have been identified and cloned. The recombinant genetic sequences of the present invention permit the modulation of expression of genes encoding this enzyme by, for example, de novo expression, over-expression, suppression, antisense inhibition and ribozyme activity. The ability to control flavonoid 3'-hydroxylase synthesis in plants permits modulation of the composition of individual anthocyanins as well as alteration of relative levels of flavonols and anthocyanins, thereby enabling the manipulation of tissue colour, such as petals,

leaves, seeds and fruit. The present invention is hereinafter described in relation to the manipulation of flower colour but this is done with the understanding that it extends to manipulation of other plant tissues, such as leaves, seeds and fruit.

#### DETAILED DESCRIPTION OF THE INVENTION

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding a flavonoid 3'-hydroxylase or a derivative thereof wherein said flavonoid 3'-hydroxylase or its derivative is capable of more efficient modulation of hydroxylation of flavonoid compounds in plants than is a flavonoid 3'-hydroxylase encoded by the nucleotide sequence set forth in SEQ ID NO:26.

Efficiency as used herein relates to the capability of the flavonoid 3'-hydroxylase enzyme to hydroxylate flavonoid compounds in a plant cell. This provides the plant with additional substrates for other enzymes of the flavonoid pathway able to further modify this molecule, via, for example, glycosylation, acylation and rhamnosylation, to produce various anthocyanins which contribute to the production of a range of colours. The modulation of 3'-hydroxylated anthocyanins is thereby permitted. Efficiency is conveniently assessed by one or more parameters selected from: extent of transcription, as determined by the amount of mRNA produced; extent of hydroxylation of naringenin and/or DHK; extent of translation of mRNA, as determined by the amount of translation product produced; extent of production of anthocyanin derivatives of DHQ or DHM; the extent of effect on tissue colour, such as flowers, seeds, leaves or fruits.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides which maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes, a flavonoid 3'-hydroxylase.

A further aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides which corresponds to the genetic locus designated Ht1 or Ht2 in petunia, or to loci in other flowering plant species which contain sequences which control production of 3'-hydroxylated flavonoids, and wherein said isolated nucleic acid molecule encodes a flavonoid 3'-hydroxylase or a derivative thereof which is capable of more efficient conversion of DHK to DHQ in plants than is the flavonoid 3'-hydroxylase set forth in SEQ ID NO:26.

In accordance with the above aspects of the present invention there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:1 under low stringency conditions.

In a related embodiment, there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:3 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:3 under low stringency conditions.

In another related embodiment, the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:5 or having at least about

60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID, NO:5 under low stringency conditions.

Yet another related embodiment provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:7 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:7 under low stringency conditions.

Still yet a further embodiment of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:9 or having at least about 60% similarity to the coding region thereof or capable of hybridising to the sequence set forth in SEQ ID NO:9 under low stringency conditions.

In another further embodiment, there is provided a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:14 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:14 under low stringency conditions.

In yet another further embodiment, the present invention is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:16 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:16 under low stringency conditions.

Still yet another further embodiment provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:18 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:18 under low stringency conditions.

Moreover, yet a further embodiment of the present invention relates to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:20 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:20 under low stringency conditions.

Still yet another further embodiment is directed to a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:22 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:22 under low stringency conditions.

In still yet another further embodiment, the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:24 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:24 under low stringency conditions.

In a particularly preferred embodiment there is provided an isolated nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence substantially as set forth in SEQ ID NO:1 or having at least about 60% similarity thereto or capable of hybridising to the sequence set forth in SEQ ID NO:1 under low stringency conditions, wherein said nucleotide sequence maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes, a flavonoid 3'-hydroxylase.

Reference herein to a low stringency at 42° C. includes and encompasses from at least about 1% to at least about 15% formamide and from at least about 1M to at least about 2M salt for hybridization, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% to at least about 30% formamide and from at least about 0.5M to at least about 0.9M salt for hybridization, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% to at least about 50% formamide and from at least about 0.01M to at least about 0.15M salt for hybridization, and at least about 0.01M to at least about 0.15M salt for washing conditions. Hybridization may be carried out at different temperatures and, where this occurs, other conditions may be adjusted accordingly.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence, having at least about 50% similarity thereto.

In a related embodiment, there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:4 or an amino acid sequence having at least about 50% similarity thereto.

A further related embodiment of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:6 or an amino acid sequence having at least about 50% similarity thereto.

Still another related embodiment provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least about 50% similarity thereto.

Yet still another related embodiment relates to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:10 or SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13 or an amino acid sequence having at least about 50% similarity thereto.

In another further embodiment, there is provided a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:15 or an amino acid sequence having at least about 50% similarity thereto.

In yet another further embodiment, the present invention is directed to a nucleic acid molecule-comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:17 or an amino acid sequence having at least about 50% similarity thereto.

Still yet another further embodiment provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:19 or an amino acid sequence having at least about 50% similarity thereto.

Moreover, yet a further embodiment of the present invention relates to: a nucleic acid molecule comprising a

sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:21 or an amino acid sequence having at least about 50% similarity thereto.

5 Still yet another further embodiment is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:23 or an amino acid sequence having at least about 50% similarity thereto.

10 In still yet another further embodiment, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:25 or an amino acid sequence having at least about 50% similarity thereto.

15 In a particularly preferred embodiment there is provided an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least about 50% similarity thereto, wherein said sequence of nucleotides maps to the genetic locus designated Ht1 or Ht2 in petunia, or to equivalent such loci in other flowering plant species, and wherein said isolated nucleic acid molecule encodes, or is complementary to a sequence which encodes, a flavonoid 3'-hydroxylase or a derivative thereof.

20 The term "similarity" as used herein includes exact identity between compared sequences, at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels.

25 The nucleic acid molecule defined by SEQ ID NO:1 encodes a flavonoid 3'-hydroxylase from petunia. Examples of other suitable F3'H genes are from carnation (SEQ ID NO:3), snapdragon (SEQ ID NO:5), arabidopsis (SEQ ID NO:7), arabidopsis genomic DNA clone (SEQ ID NO:9), rose (SEQ ID NO:14), chrysanthemum (SEQ ID NO:16), torenia (SEQ ID NO:18), Japanese morning glory (SEQ ID NO:20), gentian (SEQ ID NO:22) and lisianthus (SEQ ID NO:24). Although the present invention is particularly exemplified by the aforementioned F3'H genes, the subject invention extends to F3'H genes from any species of plant provided that the F3'H gene has at least about 60% similarity at the nucleotide level to a nucleic acid molecule selected from SEQ ID NO:1 or 3 or 5 or 7 or 14 or 16 or 18 or 20 or 22 or 24, or at least about 50% similarity at the amino acid level to an amino acid molecule selected from SEQ ID NO:2 or 4 or 6 or 8 or 10, 11, 12, 13 or 15 or 17 or 19 or 21 or 23 or 25. The subject invention further extends to F3'H genes from any species of plant provided that the F3'H gene has at least about 60% similarity at the nucleotide level to the coding region of SEQ ID NO:9.

30 The nucleic acid molecules of the present invention are generally genetic sequences in a non-naturally-occurring condition. Generally, this means isolated away from its natural state or synthesized or derived in a non-naturally-occurring environment. More specifically, it includes nucleic acid molecules formed or maintained in vitro, including genomic DNA fragments, recombinant or syn-

thetic molecules and nucleic acids in combination with heterologous nucleic acids. It also extends to the genomic DNA or cDNA or part thereof encoding F3'H or part thereof in reverse orientation relative to its or another promoter. It further extends to naturally-occurring sequences following at least a partial purification relative to other nucleic acid sequences.

The term "nucleic acid molecule" includes a nucleic acid isolate and a genetic sequence and is used herein in its most general sense and encompasses any contiguous series of nucleotide bases specifying directly, or via a complementary series of bases, a sequence of amino acids in a F3'H. Such a sequence of amino acids may constitute a full-length F3'H or an active truncated form thereof or may correspond to a particular region such as an N-terminal, C-terminal or internal portion of the enzyme. The nucleic acid molecules contemplated herein also encompass oligonucleotides useful as genetic probes or as "antisense" molecules capable of regulating expression of the corresponding gene in a plant. An "antisense molecule" as used herein may also encompass a gene construct comprising the structural genomic or cDNA gene or part thereof in reverse orientation relative to its own or another promoter. Accordingly, the nucleic acid molecules of the present invention may be suitable for use as cosuppression molecules, ribozyme molecules, sense molecules and antisense molecules to modulate levels of 3'-hydroxylated anthocyanins.

In one embodiment, the nucleic acid molecule encoding F3'H or various derivatives thereof is used to reduce the activity of an endogenous F3'H, or alternatively the nucleic acid molecule encoding this enzyme or various derivatives thereof is used in the antisense orientation to reduce activity of the F3'H. Although not wishing to limit the present invention to any one theory, it is possible that the introduction of the nucleic acid molecule into a cell results in this outcome either by decreasing transcription of the homologous endogenous gene or by increasing turnover of the corresponding mRNA. This may be achieved using gene constructs containing F3'H nucleic acid molecules or various derivatives thereof in either the sense or the antisense orientation. In a further alternative, ribozymes could be used to inactivate target nucleic acid molecules. Alternatively, the nucleic acid molecule encodes a functional F3'H and this is used to elevate levels of this enzyme in plants.

Reference herein to the altering of flavonoid F3'H activity relates to an elevation or reduction in activity of up to 30% or more preferably of 30–50%, or even more preferably 50–75% or still more preferably 75% or greater above or below the normal endogenous or existing levels of activity. The level of activity can be readily assayed using a modified version of the method described by Stotz and Forkmann (1982) (see Example 7) or by assaying for the amount of F3'H product such as quercetin, cyanidin or peonidin as set forth in Example 5.

The present invention further extends to nucleic acid molecules in the form of oligonucleotide primers or probes capable of hybridizing to a portion of the nucleic acid molecules contemplated above, and in particular those selected from the nucleic acid molecules set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 14, 16, 18, 20, 22 or 24 under high, preferably under medium and most preferably under low stringency conditions. Preferably the portion corresponds to the 5' or the 3' end of the F3'H gene. For convenience the 5' end is considered herein to define a region substantially between the 5' end of the primary transcript to a centre portion of the gene, and the 3' end is considered herein to define a region substantially between the centre portion of

the gene and the 3' end of the primary transcript. It is clear, therefore, that oligonucleotides or probes may hybridize to the 5' end or the 3' end or to a region common to both the 5' and the 3' ends.

The nucleic acid molecule or its complementary form may encode the full-length enzyme or a part or derivative thereof. By "derivative" is meant any single or multiple amino acid substitutions, deletions, and/or additions relative to the naturally-occurring enzyme and includes parts, fragments, portions, fusion molecules, homologues and analogues. In this regard, the nucleic acid includes the naturally-occurring nucleotide sequence encoding F3'H or may contain single or multiple nucleotide substitutions, deletions and/or additions to said naturally-occurring sequence. A fusion molecule may be a fusion between nucleotide sequences encoding two or more F3'Hs, or a fusion between a nucleotide sequence encoding an F3'H and a nucleotide sequence encoding any other proteinaceous molecule. Fusion molecules are useful in altering substrate specificity.

A derivative of the nucleic acid molecule or its complementary form, or of a F3'H, of the present invention may also include a "part", whether active or inactive. An active or functional nucleic acid molecule is one which encodes an enzyme with F3'H activity. An active or functional molecule further encompasses a partially-active molecule; for example, an F3'H with reduced substrate specificity would be regarded as partially active. A derivative of a nucleic acid molecule may be useful as an oligonucleotide probe, as a primer for polymerase chain reactions or in various mutagenic techniques, for the generation of antisense molecules or in the construction of ribozymes. They may also be useful in developing co-suppression constructs. The nucleic acid molecule according to this aspect of the present invention may or may not encode a functional F3'H. A "part" may be derived from the 5' end or the 3' end or a region common to both the 5' and the 3' ends of the nucleic acid molecule.

Amino acid insertional derivatives of the F3'H of the present invention include amino and/or carboxyl terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. Typical substitutions are those made in accordance with Table 1 below.

TABLE 1

Suitable residues for amino acid substitutions

Original Residue	Exemplary Substitutions
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile

TABLE 1-continued

Suitable residues for amino acid substitutions	
Original Residue	Exemplary Substitutions
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Where the F3'H is derivatised by amino acid substitution, the amino acids are generally replaced by other amino acids having like properties, such as hydrophobicity, hydrophilicity, electronegativity, bulky side chains and the like. Amino acid substitutions are typically of single residues. Amino acid insertions will usually be in the order of about 1–10 amino acid residues and deletions will range from about 1–20 residues. Preferably, deletions or insertions are made in adjacent pairs, i.e. a deletion of two residues or insertion of two residues.

The amino acid variants referred to above may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis (Merrifield, 1964) and the like, or by recombinant DNA manipulations. Techniques for making substitution mutations at predetermined sites in DNA having known or partially known sequence are well known and include, for example, M13 mutagenesis. The manipulation of DNA sequence to produce variant proteins which manifest as substitutional, insertional or deletional variants are conveniently described, for example, in Sambrook et al. (1989).

Other examples of recombinant or synthetic mutants and derivatives of the F3'H of the present invention include single or multiple substitutions, deletions and/or additions of any molecule associated with the enzyme such as carbohydrates, lipids and/or proteins or polypeptides.

The terms “analogues” and “derivatives” also extend to any chemical equivalents of the F3'H, whether functional or not, and also to any amino acid derivative described above. Where the “analogues” and “derivatives” of this aspect of the present invention are non-functional, they may act as agonists or antagonists of F3'H activity. For convenience, reference to “F3'H” herein includes reference to any derivatives, including parts, mutants, fragments, homologues or analogues thereof.

The present invention is exemplified using nucleic acid sequences derived from petunia, carnation, rose, snapdragon, arabidopsis, chrysanthemum, lisianthus, torenia, morning glory and gentian, since these represent the most convenient and preferred sources of material to date. However, one skilled in the art will immediately appreciate that similar sequences can be isolated from any number of sources such as other plants or certain microorganisms. Examples of other plants include, but are not limited to, maize, tobacco, cornflower, pelargonium, apple, gerlora and african violet. All such nucleic acid sequences encoding directly or indirectly a flavonoid pathway enzyme and in particular F3'H, regardless of their source, are encompassed by the present invention.

The nucleic acid molecules contemplated herein may exist in either orientation alone or in combination with a vector molecule, for example an expression-vector. The term vector molecule is used in its broadest sense to include any intermediate vehicle for the nucleic acid molecule, capable

of facilitating transfer of the nucleic acid into the plant cell and/or facilitating integration into the plant genome. An intermediate vehicle may, for example, be adapted for use in electroporation, microprojectile bombardment, Agrobacterium-mediated transfer or insertion via DNA or RNA viruses. The intermediate vehicle and/or the nucleic acid molecule contained therein may or may not need to be stably integrated into the plant genome. Such vector molecules may also replicate and/or express in prokaryotic cells. Preferably, the vector molecules or parts thereof are capable of integration into the plant genome. The nucleic acid molecule may additionally contain a promoter sequence capable of directing expression of the nucleic acid molecule in a plant cell. The nucleic acid molecule and promoter may also be introduced into the cell by any number of means such as those described above.

In accordance with the present invention, a nucleic acid molecule encoding a F3'H or a derivative or part thereof may be introduced into a plant in either orientation to allow, permit or otherwise facilitate manipulation of levels of production of mRNA in the cytoplasm and/or production of enzyme from the mRNA, thereby providing a means either to convert DHK and/or other suitable substrates, if synthesised in the plant cell, ultimately into anthocyanin derivatives of anthocyanidins such as cyanidin and/or peonidin, or alternatively to inhibit such conversion of metabolites by reducing or eliminating endogenous or existing F3'H activity. The production of mRNA in the cytoplasm and/or production of enzyme from the mRNA, is referred to herein as “expression”. The production of anthocyanins contributes to the production of a red or blue flower colour. Expression of the nucleic acid molecule in either orientation in the plant may be constitutive, inducible or developmental, and may also be tissue-specific.

According to this aspect of the present invention there is provided a method for producing a transgenic plant capable of synthesizing F3'H or functional derivatives thereof, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding said F3'H, under conditions permitting the eventual expression of said nucleic acid molecule, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid molecule. The transgenic plant may thereby produce elevated levels of F3'H activity relative to the amount expressed in a comparable non-transgenic plant.

Another aspect of the present invention contemplates a method for producing a transgenic plant with reduced endogenous or existing F3'H activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule which comprises a sequence of nucleotides encoding or complementary to a sequence encoding F3'H, regenerating a transgenic plant from the cell and where necessary growing said transgenic plant under conditions sufficient to permit the expression of the nucleic acid molecule.

Yet another aspect of the present invention contemplates a method for producing a genetically modified plant with reduced endogenous or existing F3'H activity, said method comprising altering the F3'H gene through modification of the endogenous sequences via homologous recombination from an appropriately altered F3'H gene or derivative or part thereof introduced into the plant cell, and regenerating the genetically modified plant from the cell.

In accordance with these aspects of the present invention the preferred nucleic acid molecules are substantially as set



forth in SEQ ID NO:1, 3, 5, 7, 14, 16, 18, 20, 22, 24 or the coding region of 9, or have at least about 60% similarity thereto, or are capable of hybridising thereto under low stringency conditions.

In a preferred embodiment, the present invention contemplates a method for producing a transgenic flowering plant exhibiting altered flower colour, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule of the present invention, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid molecule into the F3'H enzyme. Alternatively, said method may comprise stably transforming a cell of a suitable plant with a nucleic acid molecule of the present invention or its complementary sequence, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to alter the level of activity of the endogenous or existing F3'H. Preferably, the altered level would be less than the endogenous or existing level of F3'H activity in a comparable non-transgenic plant.

In a related embodiment, the present invention contemplates a method for producing a flowering plant exhibiting altered flower colour, said method comprising alteration of the F3'H gene through modification of the endogenous sequences via homologous recombination from an appropriately altered F3'H gene or derivative thereof introduced into the plant cell and regenerating the genetically modified plant from the cell.

The nucleic acid molecules of the present invention may or may not be developmentally regulated. Preferably, the modulation of levels of 3'-hydroxylated anthocyanins leads to altered flower colour which includes the production of red flowers or other colour shades depending on the physiological conditions of the recipient plant. By "recipient plant" is meant a plant capable of producing a substrate for the F3'H enzyme, or producing the F3'H enzyme itself, and possessing the appropriate physiological properties and genotype required for the development of the colour desired. This may include but is not limited to petunia, carnation, chrysanthemum, rose, snapdragon, tobacco, cornflower, pelargonium, lisianthus, gerbera, apple, iris, lily, african violet, gentian, torenia and Japanese morning glory.

Accordingly, the present invention extends to a method for producing a transgenic plant capable of modulating levels of 3'-hydroxylated anthocyanins, said method comprising stably transforming a cell or group of cells of a suitable plant with a nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding, F3'H or a derivative thereof, and regenerating a transgenic plant from said cell or cells.

One skilled in the art will immediately recognise the variations applicable to the methods of the present invention, such as increasing or decreasing the level of enzyme activity of the enzyme naturally present in a target plant leading to differing shades of colours.

The present invention, therefore, extends to all transgenic plants containing all or part of the nucleic acid module of the present invention and/or any homologues or related forms thereof or antisense forms of any of these and in particular those transgenic plants which exhibit altered flower colour. The transgenic plants may contain an introduced nucleic acid molecule comprising a nucleotide sequence encoding or complementary to a sequence encoding F3'H. Generally, the nucleic acid would be stably introduced into the plant genome, although the present invention also extends to the

introduction of the F3'H nucleotide sequence within an autonomously-replicating nucleic acid sequence such as a DNA or RNA virus capable of replicating within the plant cell. The invention also extends to seeds from such transgenic plants. Such seeds, especially if coloured, will be useful as proprietary tags for plants.

A further aspect of the present invention is directed to recombinant forms of F3'H. The recombinant forms of the enzymes will provide a source of material for research to develop, for example, more active enzymes and may be useful in developing in vitro systems for production of coloured compounds.

Still a further aspect of the present invention contemplates the use of the genetic sequences described herein in the manufacture of a genetic construct capable of use in modulating levels of 3'-hydroxylated anthocyanins in a plant or cells of a plant.

Yet a further aspect of the present invention provides flowers and in particular cut flowers, from the transgenic plants herein described, exhibiting altered flower colour.

Another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding, a F3'H or a derivative thereof wherein said nucleic acid molecule is capable of being expressed in a plant cell. The term "expressed" is equivalent to the term "expression" as defined above.

The nucleic acid molecules according to this and other aspects of the invention allow, permit or otherwise facilitate increased efficiency in modulation of 3'-hydroxylated anthocyanins relative to the efficiency of the pCGP619 cDNA insert contained in plasmid pCGP809, disclosed in International Patent Application No. PCT/AU93/00127 [WO 93/20206]. The term "plant cell" includes a single plant cell or a group of plant cells such as in a callus, plantlet or plant or parts thereof including flowers and seeds.

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence of nucleotides encoding a F3'H, wherein the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGA (SEQ ID NO:43). Preferably, the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGAXHXAYNYXDL (SEQ ID NO:44) and still more preferably the translation of the said nucleic acid molecule comprises the amino acid sequence RPPNSGAXHXAYNYXDL[X]<sub>n</sub>GGEK (SEQ ID NO:45), where X represents any amino acid and [X]<sub>n</sub> represents an amino acid sequence of from 0 to 500 amino acids.

The present invention is further described by reference to the following non-limiting Figures and Examples.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1B are schematic representations of the flavonoid biosynthesis pathways in *P. hybrida* flowers showing the enzymes and genetic loci involved in the conversions. Enzymes involved in the pathway have been indicated as follows: PAL=phenylalanine ammonia-lyase; C4H=cinnamate 4-hydroxylase; 4CL=4coumarate: CoA ligase; CHS=chalcone synthase; CHI=chalcone isomerase; F3H=flavanone 3-hydroxylase; F3'H=flavonoid 3'-hydroxylase; F3'5'H=flavonoid 3'5' hydroxylase; FLS=flavonol synthase; DFR=dihydroflavonol-4-reductase; ANS=anthocyanin synthase; 3GT=UDP-glucose: anthocyanin-3-glucoside; 3RT=UDP-rhamnose: anthocyanidin-3-glucoside rhamnosyltransferase; ACT=anthocyanidin-3-rutinoside

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acyltransferase; 5GT=UDP-glucose: anthocyanin 5-glucosyltransferase; 3' OMT=anthocyanin O-methyltransferase; 3',5' OMT=anthocyanin 3',5' O-methyltransferase. Three flavonoids in the pathway are indicated as: P-3-G=pelargonidin-3-glucoside; DHM= dihydomyricetin; DHQ=dihydroquercetin. The flavonol, myricetin, is only produced at low levels and the anthocyanin, pelargonidin, is rarely produced in *P. hybrida*.

FIG. 2 is a diagrammatic representation of the plasmid pCGP161 containing a cDNA clone (F1) representing the cinnamate-4-hydroxylase from *P. hybrida*. <sup>32</sup>P-labelled fragments of the 0.7 kb EcoRI/XhoI fragment were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp=the ampicillin resistance gene; ori=origin of replication; T3=recognition sequence for T3 RNA polymerase; T7=recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

FIG. 3 is a diagrammatic representation of the plasmid pCGP602 containing a cDNA clone (617) representing a flavonoid 3'5' hydroxylase (Hf1) from *P. hybrida*. <sup>32</sup>P-labelled fragments of the 1.6 kb BspI/EspI fragment containing the Hf1 coding region were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp=the ampicillin resistance gene; ori=origin of replication; T3=recognition sequence for T3 RNA polymerase; T7=recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

FIG. 4 is a diagrammatic representation of the plasmid pCGP175 containing a cDNA clone (H2) representing a flavonoid 3'5' hydroxylase (Hf2) from *P. hybrida*. <sup>32</sup>P-labelled fragments of the 1.3 kb EcoI/XhoI and 0.5 kb XhoI fragments which together contain the coding region were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp=the ampicillin resistance gene; ori=origin of replication; T3=recognition sequence for T3 RNA polymerase; T7=recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

FIG. 5 is a diagrammatic representation of the plasmid pCGP619 containing the 651 cDNA clone representing a cytochrome P450 from *P. hybrida*. <sup>32</sup>P-labelled fragments of the 1.8 kb EcoI/XhoI fragment were used to probe the Old Glory Red petal cDNA library. For details, refer to Example 4. Abbreviations are as follows: Amp=the ampicillin resistance gene; ori=origin of replication; T3=recognition sequence for T3 RNA polymerase; T7=recognition sequence for T7 RNA polymerase. Restriction enzyme sites are also marked.

FIG. 6 is a representation of an autoradiograph of an RNA blot probed with <sup>32</sup>P-labelled fragments of the OGR-38 cDNA clone contained in pCGP1805 (see Example 6). Each lane contained a 20 μg sample of total RNA isolated from the flowers or leaves of plants of a V23 (ht1/ht1)×VR (Ht1/ht1) backcross population. A 1.8 kb transcript was detected in the VR-like (Ht1/ht1) flowers that contained high levels of quercetin (Q+)(lanes 9–14). The same size transcript was detected at much lower levels in the V23-like (Ht1/ht1) flowers that contained little or no quercetin (Q–) (lanes 3–8). A reduced level of transcript was also detected in VR leaves (lane 1) and V23 petals (lane 2). This is described in Example 5.

FIG. 7 is a diagrammatic representation of the yeast expression plasmid pCGP1646 (see Example 7). The OGR-38 cDNA insert from pCGP1805 was cloned in a “sense”

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orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter (PGAP) in the expression vector pYE22m. TRP1=Trp1 gene, IR1=inverted repeat of, 2 μm plasmid, TGAP=terminator sequence from the yeast glyceraldehyde-3-phosphate dehydrogenase gene. Restriction enzyme sites are also marked.

FIG. 8 is a diagrammatic representation of the binary plasmid pCGP1867 (described in Example 8). The Ht1 cDNA insert (OGR-38) from pCGP1805 was closed in a “sense” orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB=left border; RB=right border; Gm=the gentamycin resistance gene; 35S=the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII=the neomycin phosphotransferase II gene; tml3'=the terminator region from the tml gene of Agrobacterium; mas3'=the terminator region from the mannopine synthase gene of Agrobacterium; ori pRi=a broad host range origin of replication from an *Agrobacterium rhizogenes* plasmid; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

FIG. 9 is a diagrammatic representation of the binary plasmid pCGP1810, preparation of which is described in Example 13. The KC-1 cDNA insert from pCGP1807 (see Example 12) was cloned in a “sense” orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB=left border; RB=right border; Gm=the gentamycin resistance gene; 35S=the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII=the neomycin phosphotransferase II gene; tml3'=the terminator region from the tml gene of Agrobacterium; mas3'=the terminator region from the mannopine synthase gene of Agrobacterium; ori pRi=a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

FIG. 10 is a diagrammatic representation of the binary plasmid pCGP1813, construction of which is described in Example 14. The KC-1 cDNA insert from pCGP1807 (see Example 12) was cloned in a “sense” orientation between the mac promoter and mas terminator. The Mac: KC-1: mas expression cassette was subsequently cloned into the binary vector pWTT2132. Abbreviations are as follows: Tet=the tetracycline resistance gene; LB=left border; RB=right border, surB=the coding region and terminator sequence from the acetolactate synthase gene; 35S=the promoter region from the cauliflower mosaic virus 35S gene, mas3'=the terminator region from the mannopine synthase gene of Agrobacterium pVS1=a broad host range origin of replication from a plasmid from *Pseudomonas aeruginosa*, pACYCori=modified replicon from pACYC184 from *E. coli*. Restriction enzyme sites are also marked.

FIG. 11 is a representation of an autoradiograph of a Southern blot probed with <sup>32</sup>P-labelled fragments of the Am3Ga differential display PCR fragment (as described in Example 16). Each lane contained a 10 μg sample of EcoRV-digested genomic DNA isolated from N8 (Eos<sup>+</sup>), K16 (eos<sup>-</sup>) or plants of an K16×N8 F<sub>2</sub> population. Hybridizing bands were detected in the genomic DNA from cyanidin-producing plants (indicated with “+”) (Lanes 1, 3, 4, 5, 6, 7, 9, 10, 12 and 15). No specific hybridization was observed in the genomic DNA samples from non-cyanidin-producing plants (indicated with “-”) (Lanes 2, 8, 11, 13 and 14).

FIG. 12 is a representation of an autoradiograph of an RNA blot probed with <sup>32</sup>P-labelled fragments of the Am3Ga

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differential display PCR fragment. Each lane contained a 10  $\mu$ g sample of total RNA isolated from the flowers or leaves of plants of an N8 (Eos<sup>+</sup>) $\times$ K16 (eos<sup>-</sup>) F<sub>2</sub> population. A 1.8 kb transcript was detected in the K16 $\times$ N8 F<sub>2</sub> flowers that produced cyanidin (cyanidin +) (plants #1, #3, #4, #5 and #8). No transcript was detected in the K16 $\times$ N8 F<sub>2</sub> flowers that did not produce cyanidin (cyanidin -) (plants #6, #11, #12) or in a leaf sample (#13L) from an K16 $\times$ N8 F<sub>2</sub> plant that produced cyanidin in the flowers. Details are provided in Example 17.

FIG. 13 is a diagrammatic representation of the binary plasmid pCGP250, construction of which is described in Example 20. The sdf3'H cDNA insert, containing the nucleotides 1 through to 1711 (SEQ ID NO:5) from pCGP246 (see Example 18), was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB=left border; RB=right border; Gm=the gentamycin resistance gene; 35S=the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII=the neomycin phosphotransferase II gene; tml3'=the terminator region from the tml gene of Agrobacterium; mas3'=the terminator region from the mannopine synthase gene of Agrobacterium; ori pRi=a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

FIG. 14 is a diagrammatic representation of the binary plasmid pCGP231, construction of which is described in Example 20. The sdf3'H cDNA insert, containing the nucleotides 104 through to 1711 (SEQ ID NO:5) from pCGP246, was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB=left border; RB=right border; Gm=the gentamycin resistance gene; 35S=the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII=the neomycin phosphotransferase II gene; tml3'=the terminator region from the tml gene of Agrobacterium; mas3'=the terminator region from the mannopine synthase gene of Agrobacterium; ori pRi=a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

FIG. 15 is a diagrammatic representation of the binary plasmid pBI-Tt7-2. The 6.5 kb EcoRI/SalI Tt7 genomic fragment from E-5 was cloned into EcoRI/SalI-cut pBI101, replacing the resident GUS gene. The orientation of the Tt7 (F3'H) gene as indicated (5' to 3') was determined through DNA sequencing. Abbreviations are as follows: LB=left border; RB=right border; nos 5'=the promoter region from the nopaline synthase gene of Agrobacterium nptII=the coding region of the neomycin phosphotransferase II gene; nos 3'=the terminator region from the nopaline synthase gene of Agrobacterium; nptII=the coding region of the neomycin phosphotransferase I gene. Restriction enzyme sites are also marked.

FIG. 16 is a diagrammatic representation of the binary plasmid pCGP2166, construction of which is described in Example 26. The rose #34 cDNA insert from pCGP2158 (see Example 25) was cloned in a "sense" orientation behind the Mac promoter in the expression vector of pCGP293. Abbreviations are as follows: LB=left border RB=right border; Gm=the gentamycin resistance gene; 35S=the promoter region from the Cauliflower Mosaic Virus 35S gene; nptII=the neomycin phosphotransferase II gene; tml3'=the terminator region from the tml gene of Agrobacterium; mas3'=the terminator region from the mannopine synthase

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gene of Agrobacterium; ori pRi=a broad host range origin of replication from a plasmid from *Agrobacterium rhizogenes*; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid. Restriction enzyme sites are also marked.

FIG. 17 is a diagrammatic representation of the binary plasmid pCGP2169 construction of which is described in Example 27. The rose #34 cDNA insert from pCGP2158 was cloned in a "sense" orientation between the CaMV35S promoter and the ocs terminator. The 35S: rose #34: ocs expression cassette was subsequently cloned into the binary vector pWTT2132. Abbreviations are as follows: Tet=the tetracycline resistance gene; LB=left border; RB=right border; surB=the boding region and terminator sequence from the acetolactate synthase gene; 35S=the promoter region from the cauliflowe mosaic virus 35S gene, ocs=terminator region from the octopine synthase gene from Agrobacterium; pVS1=a broad host range origin of replication from a plasmid from *Pseudomonas aeruginosa*, pACYCori=modified replicon from pACYC184 from *E. coli*. Restriction enzyme sites are also marked.

FIG. 18 is a diagrammatic representation of the binary plasmid pLN85, construction of which is described in Example 28. The chrysanthemum RM6i cDNA insert from pCHRM1 was cloned in "anti-sense" orientation behind the promoter from the Cauliflower Mosaic Virus 35S gene (35S). Other abbreviations are as follows: LB=left border; RB=right border; ocs3'=the terminator region from the octopine synthase gene of Agrobacterium; pnos:nptII:nos 3'=the expression cassette containing the promoter region from the nopaline synthase gene of Agrobacterium; the coding region of the neomycin phosphotransferase II gene and the terminator region from the nopaline synthase gene of Agrobacterium; oriT=origin of transfer of replication; trfA\*=a trans-acting replication function; oriColE1=a high copy origin of replication from a Colcinin E1 plasmid; Tn7SpR/StR=the spectinomycin and streptomycin resistance genes from transposon Tn7; oriVRK2=a broad host range origin of replication from plasmid RK2. Restriction enzyme sites are also marked.

FIG. 19 is a diagrammatic representation of the yeast expression plasmid pYHT6, construction of which is described in Example 30. The THT6 cDNA insert from pTHT6 was cloned in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter (PGAP) in the expression vector pYE22m. Abbreviations are as follows: TRP1=Trp1 gene; IR1=inverted repeat of 2  $\mu$ m plasmid; TGAP=the terminator sequence from the yeast glyceraldehyde-3phosphate dehydrogenase gene. Restriction enzyme sites are also marked.

FIGS. 20(i)-(v) provide a multiple sequence alignment of the predicted amino acid sequences of petunia OGR-38 (A) (SEQ ID NO:2); carnation (B) (SEQ ID NO:4); snapdragon (C) (SEQ ID NO:6); arabidopsis Tt7 coding region (D) (SEQ ID NO:42); rose (E) (SEQ ID NO:15) chrysanthemum (F) SEQ ID NO:17; torenia (G) (SEQ ID NO:19); morning glory (H) (SEQ ID NO:21); pentian (partial sequence) (I) (SEQ ID NO:23); lisianthus (partial sequence) (J) (SEQ ID NO:25) and the petunia 651 cDNA (K) (SEQ ID NO:41). Conserved amino acids are shown in bolded capital letters and are boxed and shaded. Similar amino acids are shown in capital letters and are only lightly shaded, and dissimilar amino acids are shown in lower case letters.

Amino acid abbreviations used throughout the specification are shown in Table 2, below.

TABLE 2

<u>Amino acid abbreviations</u>		
Amino acid	3-letter	single-letter
L-alanine	Ala	A
L-arginine	Arg	R
L-asparagine	Asn	N
L-aspartic acid	Asp	D
L-cysteine	Cys	C
L-glutamine	Gln	Q
L-glutamic acid	Glu	E
L-glycine	Gly	G
L-histidine	His	H
L-isoleucine	Ile	I
L-leucine	Leu	L
L-lysine	Lys	K
L-methionine	Met	M
L-phenylalanine	Phe	F
L-proline	Pro	P
L-serine	Ser	S
L-threonine	Thr	T
L-tryptophan	Trp	W
L-tyrosine	Tyr	Y
L-valine	Val	V

Table 3 provides a summary of the SEQ ID NO's assigned to the sequences referred to herein:

TABLE 3

Sequence	Species	SEQ ID NO
cDNA insert of pCGP1805	Petunia	SEQ ID NO: 1
corresponding amino acid sequence	Petunia	SEQ ID NO: 2
cDNA insert of pCGP1807	Carnation	SEQ ID NO: 3
corresponding amino acid sequence	Carnation	SEQ ID NO: 4
cDNA insert of pCGP246	Snapdragon	SEQ ID NO: 5
corresponding amino acid sequence	Snapdragon	SEQ ID NO: 6
cDNA partial sequence	Arabidopsis	SEQ ID NO: 7
corresponding amino acid sequence	Arabidopsis	SEQ ID NO: 8
genomic sequence	Arabidopsis	SEQ ID NO: 9
corresponding amino acid sequence for exon I	Arabidopsis	SEQ ID NO: 10
corresponding amino acid sequence for exon II	Arabidopsis	SEQ ID NO: 11
corresponding amino acid sequence for exon III	Arabidopsis	SEQ ID NO: 12
corresponding amino acid sequence for exon IV	Arabidopsis	SEQ ID NO: 13
cDNA insert of pCGP2158	Rose	SEQ ID NO: 14
corresponding amino acid sequence	Rose	SEQ ID NO: 15

TABLE 3-continued

Sequence	Species	SEQ ID NO
5 cDNA insert of pCHRM1	Chrysanthemum	SEQ ID NO: 16
corresponding amino acid sequence	Chrysanthemum	SEQ ID NO: 17
THT cDNA sequence	Torenia	SEQ ID NO: 18
corresponding amino acid sequence	Torenia	SEQ ID NO: 19
10 MHT 8S cDNA sequence	Jap. Morning Glory	SEQ ID NO: 20
corresponding amino acid sequence	Jap. Morning Glory	SEQ ID NO: 21
GHT13 cDNA sequence	Gentian	SEQ ID NO: 22
corresponding amino acid sequence	Gentian	SEQ ID NO: 23
15 cDNA insert of pL3-6	Lisianthus	SEQ ID NO: 24
corresponding amino acid sequence	Lisianthus	SEQ ID NO: 25
cDNA sequence from WO 93/20206	Petunia	SEQ ID NO: 26
oligonucleotide polyT-anchA		SEQ ID NO: 27
oligonucleotide polyT-anchC		SEQ ID NO: 28
oligonucleotide polyT-anchG		SEQ ID NO: 29
20 conserved amino acid primer region		SEQ ID NO: 30
corresponding oligonucleotide sequence		SEQ ID NO: 31
conserved amino acid primer region		SEQ ID NO: 32
corresponding oligonucleotide sequence		SEQ ID NO: 33
oligonucleotide primer Pet Haem-New		SEQ ID NO: 34
conserved amino acid primer region		SEQ ID NO: 35
corresponding oligonucleotide sequence		SEQ ID NO: 36
25 oligonucleotide Snapred Race A		SEQ ID NO: 37
oligonucleotide Snapred Race C		SEQ ID NO: 38
oligonucleotide poly-C Race		SEQ ID NO: 39
oligonucleotide primer Pet Haem		SEQ ID NO: 40

30 The disarmed microorganism *Agrobacterium tumefaciens* strain AGL0 separately containing the plasmids pCGP1867, pCGP1810 and pCGP231 were deposited with the Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, New South Wales, 2037. Australia on 23 Feb., 1996 and were given Accession Numbers 96/10967, 96/10968 and 96/10969, respectively.

### ISOLATION OF FLAVONOID 3'-HYDROXYLASE AND RELATED NUCLEIC ACID SEQUENCES

#### EXAMPLE 1

#### Plant Material

#### Petunia

The *Petunia hybrida* varieties used are presented in Table 4.

TABLE 4

Plant variety	Properties	Source/Reference
Old Glory Blue (OGB)	F <sub>1</sub> Hybrid	Ball Seed, USA
Old Glory Red (OGR)	F <sub>1</sub> Hybrid	Ball Seed, USA
V23	An1, An2, An3, An4, An6, An8, An9, An10, ph1, Hf1, Hf2, hfI	Wallroth et al. (1986) Doodeman et al. (1984)
R51	Rt, po, B1, F1	
	An1, An2, An3, an4, An6, An8, An9, An10, An11, Ph1, hf1, hf2,	Wallroth et al. (1986) Doodeman et al. (1984)
	HfI, rt, Po. b1, f1	
VR	V23 x 51 F <sub>1</sub> Hybrid	
SW63	An1, An2, An3, an4, An6, An8, An9, An10, An11, Ph1, Ph2, Ph5, hf1, hf2, hfI, ht2, po, mfl, f1	I.N.R.A., Dijon, Cedex France
	An1, An2, An3, An4, An6, An11,	I.N.R.A., Dijon Cedex

TABLE 4-continued

Plant variety	Properties	Source/Reference
Skr4 x SW63	hf1, hf2, htI, Ph1, Ph2, Ph5, rt, Po. Mf1, Mf2, f1 F <sub>1</sub> Hybrid	France

Plants were grown in specialised growth rooms with a 14 hour day length at a light intensity of 10,000 lux and a temperature of 22° C. to 26° C.

#### Carnation

Flowers of *Dianthus caryophyllus* cv. Korta Chanel were obtained from Van Wyk and Son Flower Supply, Victoria.

*Dianthus caryophyllus* flowers were harvested at developmental stages defined as follows:

Stage 1: Closed bud, petals not visible.

Stage 2: Flower buds opening: tips of petals visible.

Stage 3: Tips of nearly all petals exposed. "Paint-brush stage".

Stage 4: Outer petals at 45° angle to stem.

Stage 5: Flower fully open.

#### Snapdragon

The *Antirrhinum majus* lines used were derived from the parental lines K16 (eos<sup>-</sup>) and N8 (Eos<sup>+</sup>). A strict correlation exists between F3'H activity and the Eos gene which is known to control the 3'-hydroxylation of flavones, flavonols and anthocyanins (Forkmann and Stotz, 1981). K16 is a homozygous recessive mutant lacking F3'H activity while N8 is wild type for F3'H activity. These lines are similar, though not isogenic. Both parental lines and the seed from a selfed (K16×N8) F<sub>1</sub> plant were obtained from Dr C. Martin (John Innes Centre, Norwich, UK).

#### Arabidopsis

The *Arabidopsis thaliana* lines Columbia (Tt7), Landsberg erecta (Tt7) and NW88 (tt7) were obtained from the Nottingham Arabidopsis Stock Centre. Wild-type *A. thaliana* (Tt7) seeds have a characteristic brown colour. Seeds of tt7 mutants have pale brown seeds and the plants are characterized by a reduced anthocyanin content in leaves (Koornneef et al., 1982). Tt7 plants produce cyanidin while tt7 mutants accumulate pelargonidin, indicating that the Tt7 gene controls flavonoid 3'-hydroxylation.

#### Rose

Flowers of *Rosa hybrida* cv. Kardinal were obtained from Van Wyk and Son Flower Supply, Victoria.

Stages of *Rosa hybrida* flower development were defined as follows:

Stage 1: Unpigmented, tightly closed bud (10–12 mm high; 5 mm wide).

Stage 2: Pigmented, tightly closed bud (15 mm high; 9 mm wide).

Stage 3: Pigmented, closed bud; sepals just beginning to open (20–25 mm high; 13–15 mm wide)

Stage 4: Flower bud beginning to open; petals heavily pigmented; sepals have separated (bud is 25–30 mm high and 18 mm wide).

Stage 5: Sepals completely unfolded; some curling. Petals are heavily pigmented and unfolding (bud is 30–33 mm high and 20 mm wide).

#### Chrysanthemum

Stages of Chrysanthemum flower development were defined as follows:

Stage 0: No visible flower bud.

Stage 1: Flower bud visible: florets completely covered by the bracts.

Stage 2: Flower buds opening: tips of florets visible.

Stage 3: Florets tightly overlapped.

Stage 4: Tips of nearly all florets exposed; outer florets opening but none horizontal.

Stage 5: Outer florets horizontal.

Stage 6: Flower approaching maturity.

#### EXAMPLE 2

##### Bacterial Strains

The *Escherichia coli* strains used were:

DH5α supE44, Δ(lacZYA-ArgF)U169, ø80lacZΔM15, hsdR17 (r<sub>k</sub><sup>-</sup>, m<sub>k</sub><sup>+</sup>), recA1, endA1, gyrA96, thi-1, relA1, deoR (Hanahan, 1983 and BRL, 1986).

XL1-Blue MRF' Δ(mcr A)183, Δ(mcrCB-hsdSMR-mrr)173, endA1, supE44, thi-1, recA1, gyrA96, relA1, lac[F' proAB, lacIqZΔM15, Tn10(Tet<sup>r</sup>)]<sup>c</sup> (Stratagene)

XL1-Blue supE44, hsdR17 (r<sub>k</sub><sup>-</sup>, m<sub>k</sub><sup>+</sup>), recA1, endA1, gyrA96, thi-1, relA1, lac[F' proAB, lacIq, lacZΔM15, Tn10(tet<sup>r</sup>)]

SOLR e14<sup>-</sup> (mcrA), Δ(mcrCB-hsdSMR-mrr)171, sbcC, recB, recJ, umuC::Tn5(kan<sup>r</sup>), uyrC, lac, gyrA96, thi-1, relA1, [F'proAB, lacIqZΔM15], Su<sup>-</sup>(non-suppressing) (Stratagene)

DH10 B(Zip) F<sup>-</sup>mcrA, Δ(mrr-hsdRMS-mcrBC), ø80d lacZΔM15, ΔlacX74, deoR, recA1, araD139, Δ(ara, leu)7697, galU, gal|K|λ, rspL, nupG

Y1090r- ΔlacU169, (Δlon)?, araD139, strA, supF, mcrA, trpC22::Tn10Tet<sup>r</sup> [pMC9 Amp<sup>r</sup>, Tet<sup>r</sup>], mcrB, hsdR

The disarmed *Agrobacterium tumefaciens* strain AGL0 (Lazo et al., 1991) was obtained from R. Ludwig (Department of Biology, University of California, Santa Cruz, USA).

The cloning vector pBluescript was obtained from Stratagene.

Transformation of the *E. coli* strain DH5α cells was performed according to the method of Inoue et al. (1990).

#### EXAMPLE 3

##### General Methods

##### <sup>32</sup>P-Labeling of DNA Probes

DNA fragments (50 to 100 ng) were radioactively labelled with 50 μCi of [α-<sup>32</sup>P]-dCTP using an oligolabelling kit (Bresatec). Unincorporated [α-<sup>32</sup>P]-dCTP was removed by chromatography on a Sephadex G-50 (Fine) column.

##### DNA Sequence Analysis

DNA sequencing was performed using the PRISM™ Ready Reaction Dye Primer Cycle Sequencing Kits from Applied Biosystems. The protocols supplied by the manufacturer were followed. The cycle sequencing reactions were performed using a Perkin Elmer PCR machine (GeneAmp PCR System9600) and run on an automated 373A DNA sequencer (Applied Biosystems).

Homology searches against Genbank, SWISS-PROT and EMBL databases were performed using the FASTA and TFASTA programs (Pearson and Lipman, 1988) or BLAST programs (Altschul et al., 1990). Percentage sequence similarities were obtained using the LFASTA program (Pearson and Lipman, 1988). In all cases ktp values of 6 for nucleotide sequence comparisons and 2 for amino acid sequence comparisons were used, unless otherwise specified.

Multiple sequence alignments (ktp value of 2) were performed using the ClustalW program incorporated into the MaVector™6.0 application (Oxford Molecular Ltd.).

#### EXAMPLE 4

##### Isolation of a Flavonoid 3'-hydroxylase (F3'H) cDNA Done Corresponding to the Ht1 Locus from *P. hybrida* cv. Old Glory Red

In order to isolate a cDNA clone that was linked to the Ht1 locus and that represented the flavonoid 3'-hydroxylase (F3'H) in the petunia flavonoid pathway, a petal cDNA library was prepared from RNA isolated from stages 1 to 3 of Old Glory Red (OGR) petunia flowers. OGR flowers contain cyanidin based pigments and have high levels of flavonoid 3'-hydroxylase activity. The OGR cDNA library was screened with a mixture of <sup>32</sup>P-labelled fragments isolated from three cytochrome P450 cDNA clones known to be involved in the flavonoid pathway and from one cytochrome P450 cDNA clone (651) that had flavonoid 3'-hydroxylase activity in yeast. These included a petunia cDNA clone representing the cinnamtehydroxylase (C4H) and two petunia cDNA clones (coded by the Hf1 and Hf2 loci) representing flavonoid 3'5'-hydroxylase (F3'5'H) (Holton et al., 1993).

##### Construction of Petunia cv. OGR cDNA Library

Total RNA was isolated from the petal tissue of *P. hybrida* cv. OGR stage 1 to 3 flowers using the method of Turpen and Griffith (1986). Poly(A)<sup>+</sup> RNA was selected from the total RNA, using oligotex-dT™ (Qiagen).

A ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene) was used to construct a directional petal cDNA library in λZAP using 5 μg of poly(A)<sup>+</sup> RNA isolated from stages 1 to 3 of OGR as template. The total number of recombinants obtained was 2.46×10<sup>6</sup>.

After transfecting XL1-Blue MRF' cells, the packaged cDNA mixture was plated at 50,000 pfu per 15 cm diameter plate. The plates were incubated at 37° C. for 8 hours; and the phage were eluted in 100 mM NaCl, 8 mM MgSO<sub>4</sub>, 50 mM Tris-HCl pH 8.0, 0.01% (w/v) gelatin (Phage Storage Buffer (PSB)) (Sambrook et al., 1989). Chloroform was added and the phage stored at 4° C. as an amplified library.

100,000 pfu of the amplified library were plated onto NZY plates (Sambrook et al., 1989) at a density of 10,000 pfu per 15 cm plate after transfecting XL1-Blue MRF' cells, and incubated at 37° C. for 8 hours. After incubation at 4° C. overnight, duplicate lifts were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as re amended by the manufacturer.

##### Isolation of Probes

###### F3'5'H Probes

The two flavonoid 3',5' hydroxylases corresponding to the Hf1 or Hf2 loci isolated as described in Holton et al. (1993) and U.S. Pat. No. 5,349,125, were used in the screening process.

###### C4H cDNA Clones from Petunia

A number of cytochrome P450 cDNA clones were isolated in the screening process used to isolate the two

flavonoid 3',5' hydroxylase cDNA clones corresponding to the Hf1 or Hf2 loci (Holton et al., 1993; U.S. Pat. No. 5,349,125). One of these cDNA clones (F1) (contained in pCGP161) (FIG. 2) was identified as representing a cinnamate 4-hydroxylase (C4H), based on sequence identity with a previously-characterised C4H clone from mung bean (Mizutani et al., 1993). Sequence data was generated from 295 nucleotides at the 5' end of the petunia F1 cDNA clone. There was 83.1% similarity with the mung bean C4H clone over the 295 nucleotides sequenced and 93.9% similarity over the predicted amino acid sequence.

###### 651 cDNA Clone

The isolation and identification of the 651 cDNA clone contained in pCGP619 (FIG. 5) was described in the International Patent Application, having publication number W093/20206. A protein extract of yeast containing the 651 cDNA clone under the control of the yeast glyceraldehyde-3phosphate dehydrogenase promoter of pYE22m (Tanaka et al., 1988) exhibited F3'H activity.

###### Screening of OGR Library

Prior to hybridization, the duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65° C. for 30 minutes; stripped in 0.4 M sodium hydroxide at 65° C. for 30 minutes; then washed in a solution of 0.2 M Tris-HCl pH 8.0, 0.1×SSC, 0.1% (w/v) SDS at 65° C. for 30 minutes and finally rinsed in 2×SSC, 1.0% (w/v) SDS.

The lifts from the OGR cDNA library were screened with <sup>32</sup>P-labelled fragments of (1) a 0.7 kb EcoRI/XhoI fragment from pCGP161 containing the C4H cDNA clone (FIG. 2), (2) a 1.6 kb BspHI/FspI fragment from pCGP602 containing the Hf1 cDNA clone (FIG. 3), (3) a 1.3 kb EcoRI/XhoI fragment and a 0.5 kb XhoI fragment from pCGP175 containing the coding region of the Hf2 cDNA clone (FIG. 4) and (4) a 1.8 kb EcoRI/XhoI fragment pCGP619 containing the 651 cDNA clone (FIG. 5).

Hybridization conditions included a prehybridization step in 10% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42° C. for at least 1 hour. The <sup>32</sup>P-labelled fragments (each at 1×10<sup>6</sup> cpm/mL) were then added to the hybridization solution and hybridization was continued at 42° C. for a further 16 hours. The filters were then washed in 2×SSC, 1% (w/v) SDS at 42° C. for 2×1 hour and exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

Two hundred and thirty strongly hybridizing plaques were picked into PSB. Of these, 39 were rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. The plasmids contained in the λZAP bacteriophage vector were rescued and sequence data was generated from the 3' and 5' ends of the cDNA inserts. Based on sequence homology, 27 of the 39 were: identical to the petunia cinnamate 4-hydroxylase cDNA clone, 2 of the 39 were identical to the Hf1 cDNA ha clone and 7 of the 39 did not represent cytochrome P450s. The remaining 3 cDNA clones (designated as OGR-27, OGR-38, OGR-39) represented "new" cytochrome P450s, compared to the cytochrome P450 clones used in the screening procedure, and were further characterised.

#### EXAMPLE 5

##### Restriction Fragment Length Polymorphism (RFLP) Analysis

There are two genetic loci in *P. hybrida*, Ht1 and Ht2, that control flavonoid 3'-hydroxylase activity (Tabak et al., 1978;

Wiering and de Vlaming, 1984). Ht1 is expressed in both the limb and the tube of *P. hybrida* flowers and gives rise to higher levels of F3'H activity than does Ht2 which is only expressed in the tube. The F3'H is able to convert dihydrokaempferol and naringenin to dihydroquercetin and eriodictyol, respectively. In a flower producing delphinidin-based pigments, F3'H activity is masked by the F3'5'H activity. Therefore, the F3'H/F3'5'H assay (Stotz and Forkmann, 19182) is useless in determining the presence or absence of F3'H activity. The enzyme flavonol synthase is able to convert dihydrokaempferol to kaempferol and dihydroquercetin to quercetin (FIG. 1a). Myricetin, the 3',5'-hydroxylated flavonol, is produced at low levels in petunia flowers.

Therefore, analysing the flowers for the 3' hydroxylated flavonol, quercetin, infers the presence of F3'H activity.

Restriction Fragment Length Polymorphism (RFLP) analysis of DNA isolated from individual plants in a VR (Ht1/ht1)×V23 (ht1/ht1) backcross was used to determine which, if any, of the cDNA clones representing P450s were linked to the Ht1 locus. Northern analysis of RNA isolated from these plants was also used to detect the presence or absence of a transcript in these lines.

Flowers from a VR (Ht1/ht1)×V23 (ht1/ht1) backcross population were analysed for the presence of the flavonols, kaempferol and quercetin. VR (Ht1/ht1) flowers accumulate quercetin and low levels of kaempferol while V23 (ht1/ht1) flowers accumulate kaempferol but little or no quercetin. Individual plants from the VR (Ht1/ht1)×V23 (ht1/ht1) backcross were designated as VR-like (Ht1/ht1), if a substantial level of quercetin was detected in the flower extracts, and V23-like (ht1/ht1), if little or no quercetin but substantial levels of kaempferol were detected in the flower extracts (see FIG. 6).

#### Isolation of Genomic DNA

DNA was isolated from leaf tissue essentially as described by Dellaporta et al., (1983). The DNA preparations were further purified by CsCl buoyant density centrifugation (Sambrook et al., 1989).

#### Southern Blots

The genomic DNA (10 µg) was digested for 16 hours with 60 units of EcoRI and electrophoresed through a 0.7% (w/v) agarose gel in a running buffer of TAE (40 mM Tris-acetate, 50 mM EDTA). The DNA was then denatured in denaturing solution (1.5 M NaCl/0.5 M NaOH) for 1 to 1.5 hours, neutralized in 0.5 M Tris-HCl (pH 7.5)/1.5 M NaCl for 2 to 3 hours and then transferred to a Hybond N (Amersham) filter in 20×SSC.

#### RNA Blots

Total RNA was isolated from the petal tissue of *P. hybrida* cv OGR stage 1 to 3 flowers using the method of Turpen and Griffith (1986).

RNA samples were electrophoresed through 2.2 M formaldehyde/1.2% (w/v) agarose gels using running buffer containing 40 mM morpholinopropanesulphonic acid (pH 7.0), 5 mM sodium acetate, 0.1 mM EDTA (pH 8.0). The RNA was transferred to Hybond-N filters (Amersham) as described by the manufacturer.

#### Hybridization and Washing Conditions

Southern and RNA blots were probed with <sup>32</sup>P-labelled cDNA fragment (10<sup>8</sup> cpm/µg, 2×10<sup>6</sup> cpm/mL). Prehybridizations (1 hour at 42° C.) and hybridizations (16 hours at 42° C.) were carried out in 50% (v/v) formamide, 1 M NaCl, 1% (w/v) SDS, 10% (w/v) dextran sulphate. Filters were washed in 2×SSC, 1% (w/v) SDS at 65° C. for 1 to 2 hours and then 0.2×SSC, 1% (w/v) SDS at 65° C. for 0.5 to 1 hour. Filters were exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

#### RFLP and Northern Analysis of the Cytochrome P450 Fragments

RFLP analysis was used to investigate linkage of the genes corresponding to the OGR-27, OGR-38 and OGR-39 cDNA clones to the Ht1 locus.

<sup>32</sup>P-labelled fragments of OGR-27, OGR-38 and OGR-39 cDNA clones were used to probe RNA blots and Southern blots of genomic DNA isolated from individual plants in the VR×V23 backcross population. Analysis of EcoRI digested genomic DNA isolated from a VR×V23 backcross population revealed a RFLP for the OGR-38 probe which was linked to Ht1. Furthermore, a much reduced level of transcript was detected in the V23-like lines, when compared with the high levels of transcript detected in VR-like lines (FIG. 6).

The data provided strong evidence that the OGR-38 cDNA clone, contained in plasmid pCGP1805, corresponded to the Ht1 locus and represented a F3'H.

#### RFLP Analysis of a V23×R51 F<sub>2</sub> Backcross

RFLP analysis was used to investigate linkage of the gene corresponding to the OGR-38 cDNA to known genetic loci.

The RFLP linkage analysis was performed using a Macintosh version 2.0 of the MapMaker mapping program (Du Pont) (Lander et al., 1987). A LOD score of 3.0 was used for the linkage threshold.

Analysis of EcoRI or XbaI digested genomic DNA isolated from a V23×R51 F<sub>2</sub> population revealed a RFLP for the OGR-38 probe which was linked to PAC4. PAC4, a petunia actin cDNA clone (Baird and Meagher, 1987), is a molecular marker for chromosome III and is linked to the Ht1 locus (McLean et al., 1990). There was co-segregation of the OGR-38 and PAC4 RFLPs for 36 out of 44 V23×R51 F<sub>2</sub> plants. This represents a recombination frequency of 8% which is similar to a reported recombination frequency of 16% between the Ht1 locus and PAC4 (Cornu et al., 1990).

#### Further Characterisation of OGR-38

The developmental expression profiles in OGR petals, as well as in other OGR tissues, were determined by using the <sup>32</sup>P-labelled fragments of the OGR-38 cDNA insert as a probe against an RNA blot containing 20 µg of total RNA isolated from each of the five petunia OGR petal developmental stages as well as from leaves, sepals, roots, stems, peduncles, ovaries, anthers and styles. The OGR-38 probe hybridized with a 1.81 kb transcript that peaked at the younger stages of 1 to 3 of flower development. The OGR-38 hybridizing transcript was most abundant in the petals and ovaries and was also detected in the sepals, peduncles and anthers of the OGR plant. A low level of transcript was also detected in the stems. Under the conditions used, no hybridizing transcript was detected by Northern analysis of total RNA isolated from leaf, style or roots.

#### EXAMPLE 6

##### Complete Sequence of OGR-38

The complete sequence of the OGR-38 cDNA clone (SEQ ID NO:1) was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook et al., 1989). The sequence contained an open reading frame of 1536 bases which encodes a putative polypeptide of 512 amino acids.

The nucleotide and predicted amino acid sequences of OGR-38 (SEQ ID NO:1 and SEQ ID NO:2) were compared with those of the cytochrome P450 probes used in the screening process and with other petunia cytochrome P450

sequences (U.S. Pat. No. 5,349,125) using an Ifasta alignment (Pearson and Lipman, 1988). The nucleotide sequence of OGR-38 was most similar to the nucleic acid sequence of the flavonoid 3'5'-hydroxylases representing Hf1 and Hf2 loci from *P. hybrida* (Holton et al., 1993). The Hf1 clone showed 59.6% similarity to the OGR-38 cDNA clone, over 1471 nucleotides, and 49.9% similarity, over 513 amino acids, while the Hf2 clone showed 59.1% similarity to the OGR-38 cDNA clone, over 1481 nucleotides, and 49.0% similarity, over 511 amino acids.

#### EXAMPLE 7

##### The F3'H Assay of the Ht1 cDNA Clone (OGR-38) Expressed in Yeast Construction of pCGP1646

The plasmid pCGP1646 (FIG. 7) was constructed by cloning the OGR-38 cDNA insert from pCGP1805 in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter of pYE22m (Tanaka et al., 1988).

The plasmid pCGP1805 was linearised by digestion with Asp718. The overhanging 5' ends were "filled in" using DNA polymerase (Klenow fragment) according to standard protocols (Sambrook et al., 1989). The 1.8 kb OGR-38 cDNA fragment was released upon digestion with SmaI. The cDNA fragment was isolated and purified using the, Bresaclean kit (Bresatec) and ligated with blunted EcoRI ends of pYE22m. The plasmid pYE22m had been digested with EcoRI and the overhanging 5' ends were removed using DNA polymerase (Klenow fragment) according to standard protocols (Sambrook et al., 1989). The ligation was carried with the Amersham Ligation kit using 100 ng of the 1.8 kb OGR-38; fragment and 150 ng of the prepared yeast vector, pYE22m. Correct insertion of the insert in pYE22m was established by XhoI/SaII restriction enzyme analysis of the plasmid DNA <sup>1</sup>isolated from ampicillin-resistant transformants.

##### Yeast Transformation

The yeast strain G-1315 (Mat  $\alpha$ , trp1) (Ashikari et al., 1989) was transformed with pCGP1646 according to Ito et al. (1983). The transformants were selected by their ability to restore G-1315 to tryptophan prototrophy.

##### Preparation of Yeast Extracts for Assay of F3'H Activity

A single isolate of G-1315/pCGP1646 was used to inoculate 50 mL of Modified Burkholder's medium (20.0 g/L dextrose, 2.0 g/L L-asparagine, 1.5 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.5 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.33 g/L CaCl<sub>2</sub>, 2 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1 mg/L KI, 0.92 g/L (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O, 0.1 g/L nitrilotriacetic acid, 0.99 mg/L FeSO<sub>4</sub>·7H<sub>2</sub>O, 1.25 mg/L EDTA, 5.47 mg/L ZuSO<sub>4</sub>·7H<sub>2</sub>O, 2.5 mg/L FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.77 mg/L MSO<sub>4</sub>·7H<sub>2</sub>O, 0.196 mg/L CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.124 mg/L Co(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O, 10.088 mg/L Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 0.2 mg/L thiamine, 0.2 mg/L pyridoxine, 0.2 mg/L nicotinic acid, 0.2 mg/L pantothenate, 0.002 mg/L biotin, 10 mg/L inositol) which was subsequently incubated until the value at OD<sub>600</sub> was 1.8 at 30° C. Cells were collected by centrifugation and resuspended in Buffer 1 [10 mM Tris-HCl buffer (pH 7.5) containing 2 M sorbitol, 0.1 mM DTT, 0.1 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride (PMSF) and 5 mg yeast lytic enzyme/mL]. Following incubation for 1 hour at 30° C. with gentle shaking, the cells were pelleted by centrifugation and washed in ice cold Buffer 2 [10 mM Tris-HCl (pH7.5) containing 0.65 M sorbitol, 0.1 mM DTT, 0.1 mM EDTA, 0.4 mM PMSF]. The cells were then resuspended in Buffer 2 and sonicated using six 15-second bursts with a Branson Sonifier 250 at duty cycle 30% and output control 10%. The sonicated suspension was centri-

figed at 10,000 rpm for 30 minutes and the supernatant was centrifuged at 13,000 rpm for 90 minutes. The microsomal pellet was resuspended in assay buffer (100 mM potassium phosphate (pH 8), 1 mM EDTA, 20 mM 2-mercaptoethanol) and 100  $\mu$ L was assayed for activity.

##### F3'H Assay

F3'H enzyme activity was measured using a modified version of the method described by Stotz and Forkmann (1982). The assay reaction mixture typically contained 100  $\mu$ L of yeast extract, 5  $\mu$ L of 50 mM NADPH in assay buffer (100 mM potassium phosphate (pH8.0), 1 mM EDTA and 20 mM 2-mercaptoethanol) and 10  $\mu$ Ci of [<sup>3</sup>H]-naringenin and was made up to a final volume of 210  $\mu$ L with the assay buffer. Following incubation at 23° C. for 2–16 hours, the reaction mixture was extracted with 0.5 mL of ethylacetate. The ethylacetate phase was dried under vacuum and then resuspended in 10  $\mu$ L of ethylacetate. The tritiated flavonoid molecules were separated on cellulose thin layer plates (Merck Art 5577, Germany) using a chloroform:acetic acid:water (10:9:1 v/v) solvent system. The reaction products were localised by autoradiography and identified by comparison to non-radioactive naringenin and eriodictyol standards which were run alongside the reaction products and visualised under UV light.

F3'H activity was detected in extracts of G1315/pCGP1646, but not in extracts of non-transgenic yeast. From this it was concluded that the cDNA insert from pCGP1805 (OGR-38), which was linked to the Ht1 locus, encoded a F3'H.

#### EXAMPLE 8

##### Transient Expression of the Ht1 cDNA Clone (OGR-38) in Plants Construction of pCGP1867

Plasmid pCGP1867 (FIG. 8) was constructed by cloning the cDNA insert from pCGP1805 in a "sense" orientation behind the Mac promoter (Comai et al., 1990) of pCGP293 (Brugliera et al., 1994). The plasmid pCGP1805 was digested with XbaI and KpnI to release the cDNA insert. The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec) and ligated with XbaI/KpnI ends of the pCGF293 binary vector. The ligation was carried out using the Amersham ligation kit. Correct insertion of the fragment in pCGP1867 was established by XbaI/KpnI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

##### Transient Expression of the Ht1 cDNA Clone (OGR-38) in Petunia Petals

In order to rapidly determine whether the OGR-38 cDNA fragment in pCGP1867 represented a functional F3'H in plants, a transient expression study was established. Petals of the mutant *P. hybrida* line Skr4 $\times$ SW63 were bombarded with gold particles (1  $\mu$ m diameter) coated with pCGP1867 DNA.

Gold microcarriers were prewashed 3 times in 100% ethanol and resuspended in sterile water. For each shot, 1  $\mu$ g of pCGP1867 DNA, 0.5 mg of gold microcarriers, 10  $\mu$ L of 2.5 M CaCl<sub>2</sub> and 2  $\mu$ L of 100 mM spermidine (free base) were mixed by vortexing for 2 minutes. The DNA coated gold particles were pelleted by centrifugation, washed twice with 100% ethanol and finally resuspended in 10  $\mu$ L of 100% ethanol. The suspension was placed directly on the centre of the macrocarrier and allowed to dry.

Stages 1 and 2 of Skr4 $\times$ SW63 flowers were cut vertically into halves and partially embedded in MS solid media (3% (w/v) sucrose, 100 mg/L myo-inositol, 1 $\times$ MS salts, 0.5 mg/L pyridoxine-HCl, 0.1 mg/L thiamine-HCl, 0.5 mg/L nicotinic



acid and 2 mg/L glycine). The petals were placed so that the inside of the flower buds were facing upwards. A Biolistic PDS-1000/He System (Bio-Rad), using a Helium gas pressure of 900 psi and a chamber vacuum of 28 inches of mercury, was used to project the gold microcarriers into the petal tissue. After 6–12 hours under lights in a controlled plant growth room at 22° C., red anthocyanin spots were observed on the upper epidermal layer of the petal tissue bombarded with pCGP1867 ed particles. No coloured spots were observed in control petal bombarded with gold particles alone. These results indicated that the OGR-38 cDNA clone under the control of the Mac promoter was functional, at least transiently, in petal tissue.

## EXAMPLE 9

## Stable Expression of the Ht1 cDNA Clone (OGR-38) in Petunia Petals Complementation of a ht1/ht1 Petunia Cultivar

A. *tumefaciens* Transformations

The plasmid pCGP1867 (FIG. 8) was introduced into the *Agrobacterium tumefaciens* strain AGL0 by adding 5 µg of plasmid DNA to 100 µL of competent AGL0 cells prepared by inoculating a 50 mL MG/L (Garfinkel and Nester, 1980) culture and growing for 16 hours with shaking at 28° C. The cells were then pelleted and resuspended in 0.5 mL of 85% (v/v) 100 mM CaCl<sub>2</sub>/15% (v/v) glycerol. The DNA-*Agrobacterium* mixture was frozen by incubation in liquid N<sub>2</sub> for 2 minutes and then allowed to thaw by incubation at 37° C. for 5 minutes. The DNA/bacterial mix was then placed on ice for a further 10 minutes. The cells were then mixed with 1 mL of LB (Sambrook et al., 1989) media and incubated with shaking for 16 hours at 28° C. Cells of *A. tumefaciens* carrying pCGP1867 were selected on LB agar plates containing 10 µg/mL gentamycin. The presence of pCGP1867 was confirmed by Southern analysis of DNA isolated from the gentamycin-resistant transformants.

## Petunia Transformations

## (a) Plant Material

Leaf tissue from mature plants of *P. hybrida* cv Skr4×SW63 was treated in 1.25% (w/v) sodium hypochlorite for 2 minutes and then rinsed three times in sterile water. The leaf tissue was then cut into 25 mm<sup>2</sup> squares and precultured on MS media (Murashige and Skoog, 1962) supplemented with 0.05 mg/L kinetin and 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) for 24 hours.

(b) Co-cultivation of *Agrobacterium* and Petunia Tissue

*A. tumefaciens* strain AGL0 containing the binary vector pCGP1867 (FIG. 11) was maintained at 4° C. on MG/L agar plates with 100 mg/L gentamycin. A single colony was grown overnight in liquid medium containing 1% (w/v) Bacto-peptone, 0.5% (w/v) Bacto-yeast extract and 1% (w/v) NaCl. A final concentration of 5×10<sup>8</sup> cells/mL was prepared the next day by dilution in liquid MS medium containing B5 vitamins (Gamborg et al., 1968) and 3% (w/v) sucrose (BPM). The leaf discs were dipped for 2 minutes into BPM containing AGL0/pCGP1867. The leaf discs were then blotted dry and placed on co-cultivation media for 4 days. The co-cultivation medium consisted of SH medium (Schenk and Hildebrandt, 1972) supplemented with 0.05 mg/L kinetin and 1.0 mg/L 2,4-D and included a feeder layer of tobacco cell suspension spread over the co-cultivation medium with a filter paper placed on top of the tobacco cell suspension.

## (c) Recovery of Transgenic Petunia Plants

After co-cultivation, the leaf discs were transferred to selection medium (MS medium supplemented with 3% (w/v) sucrose, α-benzylaminopurine (BAP) 2 mg/L, 0.5

mg/L α-naphthalene acetic acid (NAA), kanamycin 300 mg/L, 350 mg/L cefotaxime and 0.3% (w/v) Gelrite Gellan Gum (Schweizerhall)). Regenerating explants were transferred to fresh selection medium after 4 weeks. Adventitious shoots which survived the kanamycin selection were isolated and transferred to BPM containing 100 mg/L kanamycin and 200 mg/L cefotaxime for root induction. All cultures were maintained under a 16 hour photoperiod (60 µmol. m<sup>-2</sup>, s<sup>-1</sup> cool white fluorescent light) at 23±2° C. When roots reached 2–3 cm in length the transgenic petunia plantlets were transferred to autoclaved Debco 51410/2 potting mix in 8 cm tubes. After 4 weeks, plants were replanted into 15 cm pots, using the same potting mix, and maintained at 23° C. under a 14 hour photoperiod (300 mol. m<sup>-2</sup>, s<sup>-1</sup> mercury halide light).

## EXAMPLE 10

## Transgenic Plant Phenotype Analysis

## pCGP1867 in Skr4×SW63

Table 5 shows the various petal and pollen colour phenotypes obtained with Skr4×SW63 plant transformed with the pCGP1867 plasmid. The transgenic plants #593A, 590A, 571A, 589A, 592A and 591A produced flowers with altered petal colour. Moreover, the anthers and pollen of the flowers from plants #593A, 590A, 589A, 592A and 591A were pink, compared with those of the control Skr4×SW63 plant, which were white. The change in anther and pollen colour, observed on introduction of plasmid pCGP1867 into Skr4×SW63 petunia plants, was an unanticipated outcome. The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

TABLE 5

Summary of petal, anther and pollen colours obtained in Skr4 xSW63 uz,2/31 plants transformed with pCGP1867			
Accession Number	Petal Limb Colour	RHSCC Code (petal limb)	Anther & Pollen Colour
Skr4 x SW63 control (594A)	very pale lilac	69B/73D	white
593A	dark pink	67B	pink
590A	dark pink and pink sectors	sectored 67B and 73A	pink
571A	pink	68A and B	pink
589A	dark pink	68A	pink
592A	pink and light pink sectors	68A and 68B	light pink
591A	dark pink	68A	pink
570A	very pale lilac	69B/73D	white

The expression of the introduced Ht1 cDNA in the Skr4×SW63 hybrid had a marked effect on flower colour. The stamen tissue of the non-transgenic control is white, whereas the same tissue in most of the transgenic plans was pink. In addition, expression of the Ht1 cDNA in the Skr4×SW63 hybrid conferred a dark pink hue to the corolla, which is normally very pale lilac.

## EXAMPLE 11

## Analysis of Products

The anthocyanidins and flavonols produced in the petals and stamens (included the pollen, anthers and filaments) of

the Skr4×SW63 plants transformed with pCGP 1867 were analysed by TLC.

#### Extraction of Anthocyanins and Flavonols

Prior to TLC analysis, the anthocyanin and flavonol molecules present in petal and stamen extracts were acid hydrolysed to remove glycosyl moieties from the anthocyanidin or flavonol core. Anthocyanidin and flavonol standards were used to help identify the compounds present in the floral extracts.

Anthocyanins and flavonols were extracted and hydrolysed by boiling between 100 to 200 mg of petal limbs, or five stamens, in 1 mL of 2 M hydrochloric acid for 30 minutes. The hydrolysed anthocyanins and flavonols were extracted with 200  $\mu$ L of iso-amylalcohol. This mixture was then dried down under vacuum and resuspended in a smaller volume of methanol/1% (v/v) HCl. The volume of methanol/1% (v/v) HCl used was based on the initial fresh weight of the petal so that the relative levels of flavonoids in the petals could be estimated. Extracts from the stamens were resuspended in 1  $\mu$ L of methanol/1% (v/v) HCl. A 1  $\mu$ L aliquot of the extracts from the pCGP1867 in Skr4×SW63 petals and stamens was spotted onto a TLC plate.

#### TLC Analysis of Floral Extracts

Acid-hydrolysed floral extracts were run in a Forestal solvent system (HOAc:water:HCl; 30:10:3) (Markham, 1982). Table 6 shows the results of the TLC analysis of the anthocyanidins and flavonols present in some of the flowers and stamens of the transgenic Skr4×SW63 petunia plants transformed with pCGP1867. Indicative relative amounts of the flavonols and anthocyanidins (designated with a "+" to "+++") were estimated by comparing the intensities of the spots observed on the TLC plate.

TABLE 6

Relative levels of anthocyanidins and flavonols detected in the petal limbs and stamens of Skr4 x SW63 plants transformed with pCGP1867.						
Acc #	Petal Colour	Anthocyanidins			Flavonols	
		Malvidin	Cyanidin	Peonidin	Kaempferol	Quercetin
Skr4 x SW63 control petal limb	pale lilac	+/-	-	-	+	-
593A petal limb	dark pink	-	+	+++	-	++
571A petal limb	pink	-	+	+	-	+
589A petal limb	dark pink	-	+	++	-	++
570A petal limb	pale lilac	+/-	-	-	+	-
Skr4 x SW63 control stamens	white	-	-	-	+++	+
593A stamens	pink	-	-	++	-	+++

Introduction of the Ht1 cDNA clone into Skr4×SW63 led to production of the 3'-hydroxylated flavonoids, quercetin, peonidin and some cyanidin in the petals. Peonidin is the methylated derivative of cyanidin (FIGS. 1a and 1b). Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4×SW63 control (Table 6). Although Skr4×SW63 is homozygous recessive for both the Hf1 and Hf2 genes, these mutations do not completely block production of F3'H (see U.S. Pat. No. 5,349,125) and low levels of malvidin are produced to give the petal limb a pale lilac colour.

The stamens with the pink pollen and anthers produced by the transgenic plant #593A contained peonidin and quercetin, while the non-transgenic Skr4×SW63 control with white pollen and anthers contained kaempferol and a low level of quercetin (Table 6).

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin, in the petals and stamens of the transgenic Skr4×

SW63/pCGP1867 plants correlated with the pink and dark pink colours observed in the petals, anthers and pollen of the same plants.

#### Co-suppression of F3'H Activity

The plasmid pCGP1867 was also introduced into *P. hybrida* cv. Old Glory: Red (Ht1) in order to reduce the level of F3'H activity.

Petunia transformations were carried out as described in Example 9, above.

Two out of 38 transgenic plants produced flowers with an altered phenotype. OGR normally produces deep red flowers (RHSCC#46B). The two transgenic plants with altered floral colour produced flowers with a light pink or light red hue (RHSCC#54B and #53C).

Northern analysis on RNA isolated from flowers produced by four transgenic plants (the two transgenics with an altered phenotype and two transgenics with the usual deep red flowers) was performed to examine the level of OGR-38 transcripts. Ten micrograms of total petal RNA was separated on a 1.2% (w/v) agarose/formaldehyde gel (Sambrook et al. 1989) and transferred to HybondN nylon membrane (Amersham), as described previously. Petal RNA from a non-transformed OGR flower was also included as a control. <sup>32</sup>P-labelled fragments of the OGR-38 cDNA inserts were used to probe the RNA blot.

The OGR-38 probe detected transcripts of approximately 2.4 kb and 1.8 kb in the flowers of the transgenic plants. However, the level of both transcripts detected in the light pink and light red flowers was considerably lower than that detected in the deep red transgenic flowers. The endogenous 1.8 kb transcript was also detected in RNA from the non-transformed OGR flowers. In order to confirm that the 2.4 kb

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transcript was from the introduced OGR-38 transgene, <sup>32</sup>P-labelled fragments of the mas terminator region were used to probe the same RNA blot. The mas probe detected the 2.4 kb transcript, suggesting that at least this transcript was derived from the introduced OGR-38 transgene.

#### Analysis of Anthocyanin Levels

The levels of anthocyanins in the control flowers and in the light pink transgenic flower were measured by spectrophotometric analysis.

#### Extraction of Anthocyanins and Flavonols

Anthocyanins and flavonols were extracted from petal limbs by incubating 200 to 300 mg of petal limb in 2 mL of methanol/1% (v/v) HCl for 16 hours at 4° C. Fifty  $\mu$ L of this solution was then added to 950  $\mu$ L of methanol/1% (v/v) HCl and the absorbance of the diluted solution at 530 nm was determined. The anthocyanin level in nmoles per gram

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was determined using the formula:  $[(\text{Abs}(530\text{ nm})/34,000) \times \text{volume of extraction buffer} \times \text{dilution factor} \times 10^6] / \text{weight in grams}$ .

The light pink flower was found to contain approximately 915 nmoles of anthocyanin per gram of petal limb tissue whilst the control flower contained around 4000 nmoles/gram.

These data suggest that introduction of the petunia F3'H (OGR-38) cDNA clone in a sense orientation into OGR plants leads to "co-suppression" (i.e. reduction) of both the endogenous and the transgenic F3'H transcripts. A correlation was observed between lighter flower colours, reduced anthocyanin production and reduced F3'H transcript level.

## EXAMPLE 12

Isolation of a F3'H cDNA Clone from *Dianthus caryophyllus*

In order to isolate a *Dianthus caryophyllus* (carnation) F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805 (described above), was used to screen a Carnation cv. Kortina Chanel petal cDNA library, under low stringency conditions.

Construction of Carnation cv. Kortina Chanel cDNA Library

Twenty micrograms of total RNA isolated (as described previously) from stages 1, 2 and 3 of Kortina Chanel flowers was reverse transcribed in a 50  $\mu\text{L}$  volume containing 1 $\times$ Superscript<sup>TM</sup> reaction buffer, 10 mM dithiothreitol (DTT) 500  $\mu\text{M}$  dATP, 500  $\mu\text{M}$  dGTP, 500  $\mu\text{M}$  dTTP, 500  $\mu\text{M}$  5-methyl-dCTP, 2.8  $\mu\text{g}$  Primer-Linker oligo from ZAP-cDNA Gigapack III Gold cloning kit (Stratagene) and 2  $\mu\text{L}$  Superscript<sup>TM</sup> reverse transcriptase (BRL). The reaction mix was incubated at 37° C. for 60 minutes, then placed on ice. A ZAP-cDNA Gigapack III Gold Cloning kit (Stratagene) was used to complete the library construction. The total number of recombinants was  $2.4 \times 10^6$ .

A total of 200,000 pfu of the packaged cDNA was plated at 10,000 pfu per 15 cm diameter plate after transfecting XL1-Blue MRF' cells. The plates were incubated at 37° C. for 8 hours, then stored overnight at 4° C. Duplicate lifts were taken onto Colony/Plaque Screen<sup>TM</sup> filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kortina Chanel Petal cDNA Library for a F3'H cDNA Clone

Prior to hybridization, the duplicate plaque lifts were treated as described previously. The duplicate lifts from the Kortina Chanel petal cDNA library were screened with <sup>32</sup>P-labelled fragments of the 1.8 kb EcoRI/XhoI insert from pCGP1805. Low stringency conditions, as described for the screening of the petunia OGR cDNA library, were used.

One strongly-hybridizing plaque was picked into PSB and rescreened as detailed above to isolate purified plaques. The plasmid contained in the IZAP bacteriophage vector was rescued and named pCGP1807.

The KC-1 cDNA insert contained in pCGP1807 was released upon digestion with EcoRI/XhoI and is around 2 kb. The complete sequence of the KC-1 cDNA clone was determined by compilation of sequence from subclones of the KC-1 cDNA insert. (Partial sequence covering 458 nucleotides had previously been generated from a 800 bp KpnI fragment covering the 3' region of KC-1 which was subcloned into pBluesscript to give pCGP1808.) The complete sequence (SEQ ID NO:3) contained an open reading frame of 1508 bases which encodes a putative polypeptide of 500 amino acids (SEQ ID NO:4).

The nucleotide and predicted amino acid sequences of the carnation KC-1 cDNA clone were compared with those of

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the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequences of the carnation KC-1 cDNA clone (SEQ ID NO:3 and 4) showed 67.3% similarity, over 1555 nucleotides, and 71.5% similarity, over 488 amino acids, to that of the petunia OGR-38 F3'H cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)–(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7–11 are in Example 34, at the end of the specification.

## EXAMPLE 13

## Stable Expression of the Carnation F3'H cDNA (KC-1) Clone in Petunia Petals—Complementation of a ht1/ht1 Petunia Cultivar

Preparation of pCGP1810

Plasmid pCGP1810 (FIG. 9) was constructed by cloning the cDNA insert from pCGP1807 in a "sense" orientation behind the Mac promoter (Comai et al., 1990) of pCGP90 (U.S. Pat. No. 5,349,125), a pCGP293 based construct (Brugliera et al., 1994). The plasmid pCGP1807 was digested with BamHI and ApaI to release the KC-1 cDNA insert. The cDNA fragment was isolated and purified using the Bresaclean kit (resatec). The pCGP90 binary vector was digested with BamHI and ApaI to release the linearised vector and the Hf1 cDNA insert. The linearised vector was isolated and purified using the Bresaclean kit (Bresatec) and ligated with BamHI/ApaI ends of the KC-1 cDNA clone. The ligation was carried out using the Amersham ligation. Correct insertion of the insert in pCGP1810 was established by BamHI/ApaI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

The binary vector pCGP1810 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP1810/AGL0 cells were subsequently used to transform Skr4 $\times$ SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the KC-1 cDNA clone.

## EXAMPLE 14

Transgenic Plant Phenotype Analysis pCGP1810 in Skr4 $\times$ SW63

The expression of the introduced KC-1 cDNA in the Skr4 $\times$ SW63 hybrid had a marked effect on flower colour. Ten of the twelve transgenic plants transformed with pCGP1810 produced flowers with an altered petal colour (RHSCC# 73A), compared with the Skr4 $\times$ Sw63 control (RHSCC# 75C). Moreover the anthers and pollen of the transgenic flowers were pink, compared with those of the control Skr4 $\times$ SW63 plant, which were white. In addition, expression of the KC-1 cDNA in the Skr4 $\times$ SW63 hybrid conferred a dark pink hue to the corolla, which is normally pale lilac. The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30:10:3) (Markham, 1982). The 3' hydroxylated flavonoids, peonidin

and quercetin, were readily detected in the petal limbs of the transgenic plants. Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4×SW63 control.

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin, in the petals of the transgenic Skr4×SW63/pCGP1810 plants correlated with the dark pink colours observed in the petals of the same plants.

#### Construction of pCGP1813

Plasmid pCGP1811 was constructed by cloning the cDNA insert from pCGP1807 in a "sense" orientation behind the Mac promoter (Comai et al., 1990) of pCGP1958. The plasmid pCGP1958 contains the Mac promoter and mannopine synthase (mas)(Comai et al., 1990) terminator in a pUC19 backbone. The plasmid pCGP1807 was digested with PstI and XhoI to release the cDNA insert. The overhanging 5' ends were filled in using DNA polymerase (Klenow fragment) (Sambrook et al., 1989). The cDNA fragment was isolated and purified using the Bresaclean kit (Bresatec) and ligated with SmaI ends of the pCGP1958 vector to produce pCGP1811.

The plasmid pCGP1811 was subsequently digested with PstI to release the expression cassette containing the Mac promoter driving the KC-1 cDNA with a mas terminator, all of which were contained on a 4 kb fragment. The expression cassette was isolated and ligated with PstI ends of the pWTT2132 binary vector (DNA Plant Technology, Corporation; Oakland, Calif.) to produce pCGP1813 (FIG. 10). Transformation of *Dianthus caryophyllus* cv. Kortina Chanel with the Carnation F3'H cDNA Clone.

The binary vector pCGP1813 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP1813/AGL0 cells were used to transform carnation plants, to reduce the amount of 3'-hydroxylated flavonoids.

#### (a) Plant Material

*Dianthus caryophyllus* (cv. Kortina Chanel) cuttings were obtained from Van Wyk and Son Flower Supply, Victoria, Australia. The outer leaves were removed and the cuttings were sterilised briefly in 70% v/v ethanol followed by 1.25% w/v sodium hypochlorite (with Tween 20) for 6 min and rinsed three times with sterile water. All the visible leaves and axillary buds were removed under the dissecting microscope before co-cultivation.

#### (b) Co-cultivation of Agrobacterium and Dianthus Tissue

*Agrobacterium tumefaciens* strain AGL0 (Lazo et al., 1991), containing the binary vector pCGP1813, was maintained at 4° C. on LB agar plates with 50 mg/L tetracycline. A single colony was grown overnight in liquid LB broth containing 50 mg/L tetracycline and diluted to  $5 \times 10^8$  cells/mL next day before inoculation. Dianthus stem tissue was co-cultivated with Agrobacterium for 5 days on MS medium supplemented with 3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-dichlorophenoxy-acetic acid (2,4-D), 100 mM acetosyringone and 0.25% w/v Gelrite (pH 5.7).

#### (c) Recovery of Transgenic Dianthus Plants

For selection of transformed stem tissue, the top 6–8 mm of each co-cultivated stem was cut into 3–4 mm segments, which were then transferred to MS medium (Murashige and Skoog, 1962) supplemented with 0.3% w/v sucrose, 0.5 mg/L BAP, 0.5 mg/L 2,4-D, 1 µg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite. After 2 weeks, explants were transferred to fresh MS medium containing 3% sucrose, 0.16 mg/L thidiazuron (TDZ), 0.5 mg/L indole-3-butyric acid (IBA), 2 µg/L chlorsulfuron, 500 mg/L ticarcillin and 0.25% w/v Gelrite and care was taken at this stage to remove axillary shoots from stem explants. After 3 weeks,

healthy adventitious shoots were transferred to hormone free MS medium containing 3% w/v sucrose, 5 µg/L chlorsulfuron, 500 mg/L ticarcillin, 0.25% w/v Gelrite. Shoots which survived 5 µg/L chlorsulfuron were transferred to the same medium for shoot elongation.

Elongated shoots were transferred to hormone-free MS medium containing 5 µg/L chlorsulfuron, 500 mg/L ticarcillin and 0.4% w/v Gelrite, in glass jars, for normalisation and root production. All cultures were maintained under a 16 hour photoperiod (120 mE/m<sup>2</sup>/s cool white fluorescent light) at 23±2° C. Normalised plantlets, approximately 1.5–2 cm tall, were transferred to soil (75% perlite/25% peat) for acclimation at 23° C. under a 14 hour photoperiod (200 mE/m<sup>2</sup>/s mercury halide light) for 3–4 weeks. Plants were fertilised with a carnation mix containing 1 g/L CaNO<sub>3</sub> and 0.75 g/L of a mixture of microelements plus N:P:K in the ratio 4.7:3.5:29.2.

### EXAMPLE 15

#### Isolation of a F3'H cDNA Clone from *Antirrhinum majus* (Snapdragon) Using a Differential Display Approach

A novel approach was employed to isolate a cDNA sequence encoding P3'H from *Antirrhinum majus* (snapdragon). Modified methods based on the protocols for (i) isolation of plant cytochrome P450 sequences using redundant oligonucleotides (Holton et al. 1993) and (ii) differential display of eukaryotic messenger RNA (Liang and Pardee, 1992) were combined, to compare the petal cytochrome P450 transcript populations between wild type (Eos<sup>+</sup>) and F3'H mutant (eos<sup>-</sup>)snapdragon lines. Direct cloning of differentially expressed cDNA fragments allowed their further characterisation by Northern, RFLP and sequence analysis to identify putative F3'H encoding sequences. A full-length cDNA was obtained using the RACE protocol of Frohman et al. (1988) and the clone was shown to encode a functional F3'H following both transient and stable expression in petunia petal cells.

#### Plant Material

The *Antirrhinum majus* lines used were derived from the parental lines K16 (i) and N8 (Eos<sup>+</sup>). K16 is a homozygous recessive mutant lacking F3'H activity, while N8 is wild type for F3'H activity. These lines are similar, though not isogenic. The seed of capsule E228<sup>2</sup> from the selfed K16×N8 F<sub>1</sub> plant (#E228) was germinated and the resultant plants (K16×N8 F<sub>2</sub> plants) were scored for the presence or absence of cyanidin, a product of F3'H activity (see FIGS. 1a and 1b). The presence of cyanidin could be scored visually, as the flowers were a crimson colour, unlike the mutant plants which were a pink colour (from pelargonidin-derived pigments). The accuracy of the visual scoring was confirmed by TLC analysis of the petal anthocyanins, carried out as described in Example 11.

Of 13 plants raised from the E228<sup>2</sup> seed, 9 (#3, #4, #5, #6, #7, #9, #10, #12, #15) produced flowers with cyanidin (Eos<sup>+</sup>/Eos<sup>+</sup> and Eos<sup>+</sup>/eos<sup>-</sup>) while 4 (#8, #11, #13, #14) produced only pelargonidin-derived pigments (eos<sup>-</sup>/eos<sup>-</sup>).  
Synthesis of cDNA

Total RNA was isolated from the leaves of plant #13 and petal tissue of plants #3, #5, and #12 of the *A. majus* K16×N8 F<sub>2</sub> segregating population (E228<sup>2</sup>) using the method of Turpen and Griffith (1986). Contaminating DNA was removed by treating 50 µg total RNA with 1 unit RQ1 RNase-free DNase (Promega) in the presence of 40 units RNasin® ribonuclease inhibitor (Promega) for 3 hours at 37° C. in a buffer recommended by the manufacturers. The

RNA was then further purified by extraction with phenol/chloroform/iso-amyl alcohol (25:24:1) and subsequent ethanol precipitation.

Anchored poly(T) oligonucleotides, complementary to the upstream region of the polyadenylation sequence, were used to prime cDNA synthesis from *A. majus* petal and leaf RNA. The oligonucleotide sequences synthesized were (5'-3'):

polyT-anchA	TTTTTTTTTTTTTTTTTAA	SEQ ID NO: 27
polyT-anchC	TTTTTTTTTTTTTTTTTTC	SEQ ID NO: 28
polyT-anchG	TTTTTTTTTTTTTTTTTTG	SEQ ID NO: 29

Two micrograms of total RNA and 100 pmol of the appropriate priming oligonucleotide were heated to 70° C. for 10 minutes, then chilled on ice. The RNA/primer hybrids were then added to a reaction containing 20 units RNasin® (Promega), 25 nM each DNTP 10 mM DTT and 1×Superscript buffer (BRL). This reaction was heated at 37° C. for 2 minutes, then 200 units of Superscript™ reverse transcriptase (BRL) were added and the reaction allowed to proceed for 75 minutes, after which the reverse transcriptase was inactivated by heating the mixture at 95° C. for 20 minutes.

#### Amplification of Cytochrome P450 Sequences Using PCR

Cytochrome P450 sequences were amplified using redundant oligonucleotides (designed to be complementary to conserved regions near the 3' end of plant cytochrome P450 coding sequences) and polyT anchored oligonucleotides: A similar approach was previously used to generate cytochrome P450 sequences from *Petunia hybrida* and is described in U.S. Pat. No. 5,349,125.

Four oligonucleotides (referred to as upstream primers) were synthesized. These were derived from conserved amino acid regions in plant cytochrome P450 sequences. The oligonucleotides (written 5' to 3') were as follows:

WAIGRDP	TGG GCI ATI GGI (A/C)GI GA(T/C) CC
SEQ ID NO:30	SEQ ID NO:31
FRPERF	AGG AAT T(T/C)(A/C) GIC CIG A(A/G)(A/C) GIT T
SEQ ID NO:32	SEQ ID NO:33
Pet Haem-New	CCI TT(T/C) GGI GCI GGI (A/C)GI (A/C)GI ATI TG(T/G) (C/G)CI GG
	SEQ ID NO:34
EFXPERF	GAI TT(T/C) III CCI GAI (A/C)GI TT
SEQ ID NO:35	SEQ ID NO:36

The upstream primers were used with each of the polyT anchored oligonucleotides to generate cytochrome P450 sequences in polymerase chain reactions using cDNA as a template. Fifty pmol of each oligonucleotide was combined with 2 μM of each dNTP, 1.5 mM MgCl<sub>2</sub>, 1×PCR buffer (Perkin Elmer), 5 μCi α-[P] dATP (Bresatec, 1500 Ci/mmol), 2.5 units AmpliTaq® DNA polymerase (Perkin Elmer) and 1/10th of the polyT-anchor primed cDNA reaction (from above). Reaction mixes (50 μL) were cycled 40 times between 94° C. for 15 seconds, 42° C. for 15 seconds, and 70° C. for 45 seconds, following an initial 2 minute desaturation step at 94° C. Cycling reactions were performed using a Perkin Elmer 9600 Gene Amp Thermal Cycler.

DNA sequences were amplified using each upstream primer/anchored primer combination and the appropriately-

primed cDNA template. Each primer combination was used with the cDNA from the petals of the E228<sup>2</sup> plants #3 and #5 (cyanidin-producing flowers) and #12 (non-cyanidin producing flowers). Reactions incorporating leaf cDNA from plant #13 (cyanidin-producing flowers) were also included, as negative controls, because F3'H activity is not present at a significant level in healthy, unstressed leaf tissues.

#### Differential Display of Cytochrome P450 Sequences

<sup>33</sup>P-labelled PCR fragments were visualised following separation on a 5% (w/v) polyacrylamide/urea denaturing gel (Sambrook et al. 1989). A <sup>33</sup>P-labelled M13mp18 sequencing ladder was included on the gel to serve as a size marker. The sequencing gel was dried onto Whatman 3MM paper and exposed to Kodak XAR film at room temperature.

Comparison of bands between cyanidin-producing petal samples and the non-cyanidin petal sample revealed 11 bands which represent mRNAs exclusively present in the cyanidin-producing petals. Of these 11 bands, only two were also present (at a reduced intensity) in the leaf sample.

#### Isolation and Cloning of PCR Fragments from Sequencing Gel

PCR products were purified from the dried sequencing gel and reamplified by the method described by Liang et al. (1993). Amplified cDNAs were purified, following electrophoretic separation on a 1.2% (w/v) agarose/TAE gel, using a Bresaclean kit (Bresatec). The purified fragments were then directly ligated into either commercially-prepared pCR-Script™ vector (Stratagene) or EcoRV-linearised pBluescript® (Stratagene) which had been T-tailed using the protocol of Marchuk et al. (1990).

#### Sequence of F3'H PCR Products

Each of the eleven cloned differential display PCR products (with inserts not exceeding 500 bp) was sequenced on both strands and compared to other known cytochrome P450 sequences involved in anthocyanin biosynthesis, using the FASTA algorithm of Pearson and Lipman (1988).

Of the eleven cDNAs cloned, two (Am1Gb and Am3Ga) displayed strong homology with the petunia OGR-38 F3'H

sequence (Examples 4 to 11) and the F3'5'H sequences (Holton et al., 1993). Conserved sequences between clones Am1Gb and Am3Ga suggested that they represented overlapping fragments of the same mRNA. Clone Am3Ga extends from the sequence encoding the haem-binding region of the molecule (as recognised by the "Pet Haem-New" oligonucleotide; SEQ ID NO:34) to the polyadenylation sequence. Clone Am1Gb extends from the cytochrome P450 sequence encoding the conserved "WAIGRDP" amino acid motif (complementary to primer 1; SEQ ID NO:30 and SEQ ID NO:31) to an area in the 3' untranslated region which was spuriously recognised by the primer 1 ("WAIGRDP") oligonucleotide.

#### EXAMPLE 16

##### RFLP Analysis of Cytochrome P450 cDNAs

Restriction fragment length polymorphism (RFLP) analysis was again used to investigate linkage of the gene corre-

sponding to cDNA clone Am3Ga to the presence, or absence, of cyanidin-producing activity in petals. A <sup>32</sup>P-labelled insert of Am3Ga was used to probe Southern blots of genomic DNA isolated from K16×N8 F<sub>2</sub> segregating plants as well as the parental K16 and N8 lines. Analysis of EcoRV-digested genomic DNA from 13 plants of the K16×N8 F<sub>2</sub> segregating population revealed hybridization only to the sequences of N8 and the K16×N8 F<sub>2</sub> segregating lines which displayed floral cyanidin production (FIG. 11). The K16×N8 F<sub>2</sub> plants which produced only pelargonidin-derived pigments in their petals (including parental line, K16) showed no specific hybridization (FIG. 11, lanes 2, 8, 11, 13, 14). These data indicate a possible deletion of the genomic sequences corresponding to Am3Ga in the mutant K16 plant and, therefore, at least a partial deletion of the F3'H gene in this line.

## EXAMPLE 17

## Northern Analysis of Cytochrome P450 cDNAs

Northern analysis was used to confirm the expression profiles of the putative cytochrome P450 fragments as shown by differential display. Ten micrograms of total petal RNA from eight of the K16×N8 F<sub>2</sub> segregating population was separated on a 1.2% (w/v) agarose/formaldehyde gel (Sambrook et al. 1989) and transferred to HybondN nylon membrane (Amersham). Leaf RNA from the cyanidin-producing plant #13 was also included as a negative control in the Northern analysis. <sup>32</sup>P-labelled fragments of the cDNA insert from clone Am3Ga was used to probe the RNA blot.

The Am3Ga probe recognised an approximately 1.8 kb transcript which was only detectable in the petals of cyanidin-producing plants (plants #1, #3, #4, #5, #8). No transcript was detectable in the pelargonidin-producing petals (plants #6, #11, #12) or in the leaf sample from plant #13 (FIG. 12).

These data, taken with those of the RFLP analysis, provide strong evidence that Am3Ga clone represents a cytochrome P450 gene which is responsible for F3'H activity in snapdragon. The total lack of a detectable transcript in the petals of non cyanidin-producing lines supports the findings of the RFLP analysis, that the loss of cyanidin-producing activity in the K16 line (and the homozygous recessive plants of the K16×N8 F<sub>2</sub> segregating population) is the result of a deletion in the F3'H structural gene.

## EXAMPLE 18

## Isolation of a Full-length Snapdragon F3'H cDNA

The Rapid Amplification of cDNA Ends (RACE) protocol of Frohman et al (1988) was employed to isolate a full-length F3'H cDNA clone using sequence knowledge of the partial Am3Ga clone. A gene-specific primer ("SnapredRace A"—complementary to Am3Ga sequences 361 to 334) was synthesized to allow reverse transcription from petal RNA. A 3' amplification primer ("SnapredRace C"—complementary to Am3Ga (3'UTR) sequences 283 to 259) was also synthesized to bind just upstream of "SnapredRace A". A "poly(C)" primer was used to amplify sequences from the 5' end of the cDNA molecule.

The sequences of the oligonucleotides used were (written 5'-3'):

5	Snapred	CCA CAC GAG TAG TTT TGG CAT TTG ACC C
	Race A	SEQ ID NO:37
	Snapred	GTC TTG GAC ATC ACA CTT CAA TCT G
	Race C	SEQ ID NO:38
10	PolyC	CCG AAT TCC CCC CCC CC
	race	SEQ ID NO:39

"Snapred Race A-primed" petal cDNA was poly(G)-tailed and a 5' cDNA fragment amplified with primers "Snapred Race C" and "PolyC race" using the method of Frohman et al. (1988). Pfu DNA polymerase (0.15 unit) (Stratagene) was combined with 2.5 units AmpliTaq® DNA polymerase (Perkin Elmer) to increase the fidelity of the PCR reaction.

The resultant 1.71 kb DNA fragment (sdF3'H) was cloned directly into EcoRV-linearised pBluescript® (Stratagene) vector which had been T-tailed using the protocol of Marchuk et al. (1990). This plasmid was named pCGP246.

## EXAMPLE 19

## Complete Sequence of Snapdragon F3'H

Convenient restriction sites within the sdF3'H cDNA sequence of pCGP246 were exploited to generate a series of short overlapping subclones in the plasmid vectors pUC19. The sequence of each of these subclones was compiled to provide the sequence of the entire sdF3'H RACE cDNA. The sdF3'H cDNA sequence was coupled with that from clone Am3Ga to provide the entire sequence of a snapdragon F3'H cDNA (SEQ ID NO:5). It contains an open reading frame of 1711 bases which encodes a putative polypeptide of 512 amino acids (SEQ ID NO:6).

The nucleotide and predicted amino acid sequences of the snapdragon sdF3'H clone were compared with: those of the petunia OGR-38 cDNA clone (SEQ ID NO:1 and SEQ ID NO:2); the petunia F3'5'H cDNA clones Hf1 and Hf2; and other petunia cytochrome P450 sequences isolated previously (U.S. Pat. No. 5,349,125). The sequence of sdF3'H was most similar to that of the petunia F3'H cDNA clone (OGR-38) representing the Ht1 locus from *P. hybrida*, having 69% similarity at the nucleic acid level, over 1573 nucleotides, and 72.2% similarity at the amino acid level, over 507 amino acids.

The Hf1 clone showed 57.3% similarity, over 1563 nucleotides and 49.3% similarity, over 491 amino acids, to the snapdragon sdF3'H clone, while the Hf2 clone showed 57.7% similarity, over 1488 nucleotides, and 50.8% similarity, over 508 amino acids, to the snapdragon sdF3'H clone.

The snapdragon sdF3'H sequence contains two "in frame" ATG codons which could act to initiate translation. Initiation from the first of these codons (position 91 of SEQ ID NO:5) gives a protein with an additional 10 N-terminal amino acids and would be favoured according to the scanning model for translation (Kozak, 1989).

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among

the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)–(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7–11 are in Example 34, at the end of the specification.

## EXAMPLE 20

Transient Expression of sdF3'H in Plants  
Constriction of pCGP250

Plasmid pCGP250 (FIG. 13) was created by cloning the entire sdF3'H RACE cDNA insert (from position 1 to 1711 (SEQ ID NO:5)) from pCGP246 in the “sense” orientation behind the Mac promoter (Comai et al., 1990) of pCGP293 (Brugliera et al., 1994). The plasmid pCGP246 was digested with EcoRI to release the cDNA insert. The cDNA fragment was blunt-ended by repairing the overhangs with the Klenow fragment of DNA polymerase I (Sambrook et al., 1989) and purified, following agarose gel electrophoresis, using a Bresaclean kit (Bresatec). The blunt cDNA fragment was then ligated into the binary vector pCGP293, which had been linearised with XbaI and blunt-ended using the Klenow fragment of DNA polymerase I. The ligation was carried out using the Amersham ligation kit. Correct insertion of the insert in pCGP250 was established by BamHI and PstI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

## Construction of pCGP231

Plasmid pCGP231 (FIG. 14) was created by cloning the RACE cDNA insert from pCGP246, downstream of the first “in-frame” ATG codon (from position 104 to 1711 (SEQ ID NO:5), in the “sense” orientation behind the Mac promoter (Comai et al., 1990) of pCGP293 (Brugliera et al., 1994). The plasmid pCGP246 was digested with SspI (which recognises a site between the candidate ATG codons) and SmaI (with a site in the vector polylinker sequence) to release a blunt-ended cDNA fragment which includes the entire coding region downstream from the second putative initiation codon. The cDNA fragment was then ligated into the binary vector pCGP293, which had been linearised with XbaI and blunt-ended using the Klenow fragment of DNA polymerase I. The ligation was carried out using the Amersham ligation kit. Correct insertion of the insert in pCGP231 was established by BamHI and PstI restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

## Transient Expression Studies

To determine rapidly whether the pCGP246 sequences in pCGP231 and pCGP250 encoded active flavonoid 3'-hydroxylases in plants, a transient expression study was undertaken. Petals of the mutant *P. hybrida* line Skr4×SW63 were bombarded with gold particles (1 μm diameter) coated with either pCGP231 or pCGP250 plasmid DNA, using the method described in Example 8.

After 6–12 hours under lights in a controlled plant growth room at 22° C., red anthocyanin spots were observed on the surface of the petal tissue bombarded with pCGP231 coated particles. No coloured spots were observed in petals bombarded with pCGP250 or control petals bombarded with gold particles alone. These results indicated that the pCGP246 coding region (starting at the second ATG, position 121 of SEQ ID NO:5), under the control of the Mac promoter, was functional in petal tissue.

## EXAMPLE 21

Stable Expression of the Snapdragon F3'H cDNA  
Clone in Petunia Petals—Complementation of a  
ht1/ht1 Petunia Cultivar

The binary vectors pCGP250 and pCGP231 were introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP250/AGL0 and pCGP231/AGL0 cells were used to transform Skr4×SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the snapdragon sdF3'H cDNA clone.

Three of the nine transgenic plants transformed with pCGP250 produced flowers with a slightly-altered petal colour (RHSCC# 73A), compared with the Skr4×Sw63 control (RHSCC# 75C). Of the 11 transgenic plants transformed with pCGP231, one plant produced flowers with an altered petal colour (RHSCC# 73B). The anthers and pollen of the transgenic flowers were also white, as in the control. The codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

## TLC Analysis of Floral Extracts

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30:10:3) (Markham, 1982). Introduction of the sdF3'H cDNA clone into Skr4×SW63 led to the production of increased levels of the 3'-hydroxylated flavonoid, peonidin, in the petals. Peonidin is the methylated derivative of cyanidin (FIGS. 1a and 1b).

## EXAMPLE 22

Isolation of a F3'H cDNA Clone from *Arabidopsis thaliana* Using a PCR Approach

In order to isolate a cDNA clone representing flavonoid 3'-hydroxylase from *Arabidopsis thaliana*, PCR fragments were generated using primers from the conserved regions of cytochrome P450s. One PCR product (p58092.13) was found to have high sequence similarity with the petunia OGR-38 and snapdragon F3'H cDNA clones. The PCR fragment was then used, together with the Ht1 cDNA insert (OGR-38) from pCGP 805, to screen an *A. thaliana* cDNA library.

## Design of Oligonucleotides

Degenerate oligonucleotides for PCR DNA amplification were designed from the consensus amino acid sequence of *Petunia hybrida* cytochrome P450 partial sequences situated near the haem-binding domain. Primer degeneracy was established by the inclusion of deoxyinosine (designated as I below) in the third base of each codon (deoxyinosine base pairs with similar efficiency to A, T, G, and C), and the inclusion of alternate bases where the consensus sequences were non-specific. Thus, the amino-terminal directional primer “Pet Haem” (*Petunia* haem-binding domain), containing the cysteine residue codon crucial for haem binding, and the upstream primer “WAIGRDP” (See also Example 15) were designed.

WAIGRDP TGG GCI ATI GGI (A/C)GI GA(T/C) CC  
SEQ ID NO:30 SEQ ID NO:31

Pet Haem CCI GG(A/G) CAI ATI C(G/T)(C/T) (C/T)TI CCI GCI CC(A/G) AAI GG  
SEQ ID NO:40

#### Generation of Cytochrome P450 Sequences Using PCR

Genomic DNA was isolated from *A. thaliana* ecotype Columbia, using the method described by Dellaporta et al. (1987). Polymerase chain reactions for amplification of cytochrome P450 homologues typically contained 100–200 ng of Columbia genomic DNA, 10 mM Tris-HCl (pH8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.01% (w/v) gelatin, 0.2 mM each DNTP, 312 ng “WAIGRDP” and 484 ng “Pet Haem” and 1.25 units Taq polymerase (Cetus). Reaction mixes (50  $\mu$ L) were cycled 40 times between 95° C. for 50 seconds, 45° C. for 50 seconds and 72° C. for 45 seconds.

The expected size of specific PCR amplification products, using the “WAIGRDP” and “Pet Haem” primers on a typical P450 gene template, without an intron, is approximately 150 base pairs. PCR fragments of approximately 140 to 155 base pairs were isolated and purified using the Mermaid® kit (BIO 101). The PCR fragments were re-amplified to obtain enough product for cloning and then end-repaired using Pfu DNA polymerase and finally cloned into pCR-Script™ Direct SK(+) (Stratagene). The ligated DNA was then used to transform competent DH5 $\alpha$  cells (Inoue et al., 1990).

#### Sequence of PCR Products

Plasmid DNA from 15 transformants was prepared (Del Sal et al., 1989). Sequencing data generated from these PCR fragments indicated that 11 out of the 15 represented unique clones. A distinct set of cytochrome P450 consensus amino acids was also found in the translated sequence encoded within the *A. thaliana* PCR inserts. The sequences of the PCR fragments were also compared with those of the petunia OGR-38 F3'H cDNA clone and the snapdragon F3'H cDNA clone. The PCR fragment, p58092.13, was most similar to the F3'H sequences from both petunia and snapdragon.

### EXAMPLE 23

#### Screening of *A. thaliana* cDNA Library

To isolate a cDNA clone of the p58092.13 PCR product, an *A. thaliana* ecotype Columbia cDNA library (Newman et al., 1994; D'Alessio et al., 1992) was screened with a <sup>32</sup>P-labelled fragment of p58092.13 together with a <sup>32</sup>P-labelled fragment of the petunia Ht1 cDNA insert (OGR-38), contained in pCGP1805.

A total of 600,000 pfu was plated at a density of 50,000 pfus per 15 cm diameter plate, as described by D'Alessio et al. (1992). After phage growth at 37° C. plates were stored at 4° C. overnight, duplicate lifts were taken onto Colony/Plaque Screen filters (DuPont) and treated as recommended by the manufacturer.

Prior to hybridization, the duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65° C. for 30 minutes; stripped in 0.4 M sodium hydroxide at 65° C. for 30 minutes; then washed in a solution of 0.2 M Tris-HCl pH 8.0, 0.1 $\times$ SSC, 0.1% (w/v) SDS at 65° C. for 30 minutes and finally rinsed in 2 $\times$ SSC, 1.0% (w/v) SDS.

Hybridization conditions included a prehybridization step in 50% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran

sulphate, 1% (w/v) SDS at 42° C. for at least 1 hour. The <sup>32</sup>P-labelled fragment of p58092.13 (2 $\times$ 10<sup>6</sup> cpm/mL) was then added to the hybridization solution and hybridization was continued at 42° C. for a further 16 hours. The filters were then washed in 2 $\times$ SSC, 1% (w/v) SDS at 42° C. for 2 $\times$ 1 hour and exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

Eleven strongly-hybridizing plaques were picked into PSB and rescreened as detailed above, to isolate purified plaques. These filters were also probed with <sup>32</sup>P-labelled fragment of the petunia Ht1 cDNA insert (OGR-38), contained in pCGP1805, under low stringency conditions. Low stringency conditions included prehybridization and hybridization at 42° C. in 20% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS and washing in 6 $\times$ SSC, 1% (w/v) SDS (w/v) at 65° C. for 1 hour.

The OGR-38 and p58092.13 probes hybridized with identical plaques. The 11 pure plaques were picked into PSB and the plasmid vectors pZL1 containing the cDNA clones were rescued using the bacterial strain DH10B(Zip). Plasmid DNA was prepared (Del Sal et al., 1989) and the cDNA inserts were released upon digestion with BamHI and EcoRI. The 11 plasmids contained cDNA inserts of between 800 bp and 1 kb. Sequence data generated from the 5' region of the cDNA inserts suggested that nine of these clones were identical. Sequence data were generated from the 5' ends of all nine cDNA inserts and the 3' end of only one cDNA insert. The sequence data generated from all clones were compiled to produce the nucleotide and translated sequence shown as SEQ ID NO:7 and SEQ ID NO:8.

The *A. thaliana* putative F3'H sequences were compared with the sequences of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2) and was 64.7% similar to the petunia F3'H cDNA clone, over 745 nucleotides, and 63.7% similar, over 248 amino

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)–(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7–11 are in Example 34, at the end of the specification.

#### Isolation of a F3'H Genomic Clone from *Arabidopsis thaliana*

To isolate a genomic clone of the *A. thaliana* F3'H gene, a *A. thaliana* ecotype Landsberg erecta genomic DNA library was screened with <sup>32</sup>P-labelled p60606.04 fragments. The library was created by cloning partial MboI-digested genomic DNA between BamHI-digested bacteriophage lambda EMBL4 arms. The primary library, which contained 30,000 clones, was amplified once before screening.

The p60606.04 clone, containing a 1 kb fragment of *A. thaliana* F3'H cDNA, was digested with BamHI/EcoRI to excise the insert which was purified using GeneClean (Bio 101). Probe was <sup>32</sup>P-labelled using the nick-translation procedure (Sambrook et al., 1989). Approximately 20,000 plaques were probed at high stringency (50% formamide at



37° C.) and filters were washed in: 2×SSPE; 2×SSPE, 0.1% (w/v) SDS; 0.1×SSPE, all at 65° C. Re-screening was carried out under the same conditions.

DNA was purified from three positive plaques ( $\lambda$ TT7-1,  $\lambda$ TT7-5 and  $\lambda$ TT7-6) and mapped by digestion with EcoRI and EcoRI/SalI. All three clones had an EcoRI fragment in common.  $\lambda$ TT7-1 and  $\lambda$ TT7-5 had overlapping but not identical restriction patterns. A Southern blot of these digests was probed as above and, for  $\lambda$ TT7-1 and  $\lambda$ TT7-5, a common 6.5 kb EcoRI/SalI fragment hybridized. A smaller EcoRI/SalI fragment in  $\lambda$ TT7-6 also hybridized and was presumably at the insert boundary.

EcoRI/SalI fragments from ITT7-5 were cloned into pBlueScript SK+ and a clone containing the 6.5 kb fragment, designated E-5, was identified by hybridization (as above) and insert size. A restriction map was compiled for the fragment using EcoRI, SalI, KpnI, HindIII and BglII in various combinations, and by hybridization to Southern blots of these digests with the BamHI/EcoRI insert from the *A. thaliana* F3'H cDNA clone.

#### Complete Sequence of Tt7 Genomic Clone

A 6.4 kb BamHI fragment from pTt7-2, containing most of the Tt7 genomic fragment was purified, self-ligated, sonicated, end-repaired, size-fractionated (450 bp to 800 bp) and cloned into SmaI-cut pUC19 using standard techniques (Sambrook et al., 1989). Recombinant clones were isolated, and plasmid DNA was purified and sequenced using M13-21 or M13 reverse sequencing primers. The sequence from overlapping clones was combined into one contiguous fragment. The sequence of the ends of the Tt7 genomic fragment were also obtained by sequencing with the -21 and REV primers. All of the sequences were combined together to obtain the complete sequence of the 6.5 kb EcoRI/SalI fragment from E-5 (SEQ ID NO:9).

The sequences over the coding region of the arabidopsis Tt7 genomic clone (SEQ ID NO:10, 11, 12 and 13) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and 2). The arabidopsis Tt7 coding region showed 65.4% similarity, over 1066 nucleotides, and 67.1% similarity, over 511 amino acids, to that of the petunia OGR-38 F3'H cDNA clone.

#### Transformation of a tt7 Arabidopsis Mutant

##### Preparation of Binary Vector

The EcoRI/SalI fragment from E-5 was cloned into EcoRI/SalI-cut pBI101 (Jefferson et al., 1987). Two separate but identical clones were identified: pBI-Tt7-2 (FIG. 15) and pBI-Tt7-4. Both clones were used for transformation of *A. tumefaciens*.

##### Plant Transformation

Plasmids pBI-Tt7-2, pBI-Tt7-4 and pBI101 were transformed into *Agrobacterium* strain GV3101 pMP90 by electroporation. Transformants were selected on medium containing 50  $\mu$ g/mL kanamycin (and 50  $\mu$ g/mL gentamycin to select for the resident pMP90).

Plasmid DNA, from four transformant colonies for each clone, was isolated and digested with EcoRI/SalI, electrophoresed, Southern blotted, and probed with the Tt7 cDNA insert. For pBI-Tt7-2 and pBI-Tt7-4, the expected insert band was identified.

One transformant for each plasmid (i.e.: one control [PBI101 C4], one each of the two Tt7 clones [pBI-Tt7-2-3 and pBI Tt7-4-4]) was used to vacuum infiltrate the *A. thaliana* tt7 mutant line NW88 (4 pots of 10 plants each for each construct), using the a method essentially as described by Bechtold et al. (1993).

Seed from each pot was harvested. One hundred mg of seed (approximately 5,000) was plated on nutrient medium

(described by Haughn and Somerville, 1986) containing 50  $\mu$ g/mL kanamycin. Kanamycin-resistant transformants were visible after 7 to 10 days. In the case of pBI-Tt7-2-3 and pBI-Tt7-4-4, a total of 11 transformants were isolated from 5 different seed lots (i.e.: pots) and all kanamycin-resistant transformants were visibly Tt7 in phenotype and exhibited the characteristic red/purple anthocyanin pigments at the margins of the cotyledons and at the hypocotyl. A single kanamycin-resistant transformant was isolated from only one of the four pots of control transformants and it did not exhibit a "wild-type" Tt7 phenotype.

#### Complementation of tt7 Mutant

These transformants were planted out and grown to maturity and individually harvested for seed. In each case, for pBI-Tt7-2-3 and pBI-Tt7-4-4 transformants, the seeds were visibly more brown than the pale brown seed of the tt7 mutant plants. The seed from the control transformant was indistinguishable from the tt7 mutant parent. These seed were plated out on nutrient medium and nutrient medium with kanamycin added, and scored for the Tt7 phenotype (red/purple anthocyanin pigments at the margins of the cotyledons and at the hypocotyl) and kanamycin resistance. The progeny of at least one transformant for each seed lot was examined, since these were clearly independent transformation events.

Without exception, kanamycin-resistant seedlings exhibited the Tt7 phenotype while kanamycin-sensitive individuals were tt7. In some cases, kanamycin resistance was weak and variable among a family of seed and it was difficult to unequivocally determine whether individuals were kanamycin resistant or kanamycin sensitive.

#### EXAMPLE 24

##### Isolation of a F3'H cDNA Clone from *Rosa hybrids*

In order to isolate a Rose F3'H cDNA clone, a *Rosa hybrida* cv. Kardinal petal cDNA library was screened with <sup>32</sup>P-labelled fragments of the petunia Ht1 cDNA clone (OGR-38), contained in pCGP1805, and snapdragon F3'H cDNA clone (sdF3'H), contained in pCGP246.

##### Construction of a Petal cDNA Library from Rose cv. Kardinal

Total RNA was prepared from the buds of *Rosa hybrida* cv. Kardinal stage 2. At this stage, the tightly closed buds were 1.5 cm high and approximately 0.9 cm wide with pale pink petals.

Frozen tissue (1-3 g) was ground in liquid nitrogen with a mortar and pestle, placed in 25 mL pre-chilled Buffer A [0.2 M boric acid, 10 mM EDTA (sodium salt) (pH 7.6)] and homogenized briefly. The extract was mixed on a rotary shaker until it reached room temperature and an equal volume of phenol/chloroform (1:1 v/v), equilibrated with Buffer A, was added. After mixing for a further 10 minutes, the RNA preparation was centrifuged at 10,000×g for 10 minutes at 20° C. The upper aqueous phase was retained and the phenol interface re-extracted as above. The aqueous phases were pooled and adjusted to 0.1 M sodium acetate (pH 6.0), 2.5 volumes 95% ethanol were added and the mixture was stored at -20° C. overnight.

The preparation was centrifuged at 10,000×g for 10 minutes at 4° C., the pellet dissolved gently in 20 mL Buffer B [25 mM boric acid, 1.25 mM EDTA (sodium salt), 0.1 M NaCl (pH 7.6)] and 0.4 volumes 2-butoxyethanol (2BE) were added. This solution was incubated on ice for 30 minutes. It was then centrifuged at 10,000×g for 10 minutes at 0° C. and the supernatant was carefully collected. After addition of 1.0 volume of 2BE and incubation on ice for a

further 30 minutes, the supernatant was again centrifuged at 10,000×g for 10 minutes at 0° C. The resulting pellet was gently washed with Buffer A:2BE (1:1 v/v), then with 70% (v/v) ethanol, 0.1 M potassium acetate and finally with 95% ethanol. The pellet was air dried and dissolved in 1 mL diethyl pyrocarbonate (DEPC)-treated water. This was adjusted to 3 M lithium chloride, left on ice for 60 minutes and centrifuged at 10,000×g for 10 minutes at 0° C. The pellet was washed twice with 3 M LiCl and then with 70% ethanol, 0.1 M potassium acetate.

The resulting RNA pellet was dissolved in 400 μL DEPC-treated water and extracted with an equal volume phenol/chloroform. The RNA mix was then centrifuged at 10,000×g for 5 minutes at 20° C., the aqueous phase collected and made to 0.1 M sodium acetate, and a further 2.5 volumes of 95% ethanol were added. After 30 minutes incubation on ice, the mix was centrifuged at 13,000 rpm (5,000×g) for 20 minutes at 20° C. and the RNA pellet resuspended gently in 400 μL DEPC-treated water.

Poly (A)<sup>+</sup> RNA was selected from the total RNA by Oligotex dT-30 (Takara, Japan) following the manufacturer's protocol. The cDNA was synthesized according to the method in Brugliera et al. (1994) and used to construct a non-directional petal cDNA library in the EcoRI site of λZAPII (Stratagene). The total number of recombinants obtained was 3.5×10<sup>5</sup>.

After transfecting XL1-Blue cells, the packaged cDNA mixture was plated at 50,000 pfu per 15 cm diameter plate. The plates were incubated at 37° C. for 8 hours, and the phage were eluted in 100 mM NaCl, 8 mM MgSO<sub>4</sub>, 50 mM Tris-HCl pH 8.0, 0.01% (w/v) gelatin (Phage Storage Buffer (PSB)) (Sambrook et al., 1989). Chloroform was added and the phage stored at 4° C. as an amplified library.

200,000 pfus of the amplified library were plated onto NZY plates (Sambrook et al., 1989) at a density of 10,000 pfu per 15 cm plate after transfecting XL1-Blue MRF' cells, and incubated at 37° C. for 8 hours. After incubation at 4° C. overnight, duplicate lifts (labelled as group A and group B) were taken onto Colony/Plaque Screen filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kardinal cDNA Library for a F3'H cDNA Clone

Prior to hybridization, The duplicate plaque lifts were washed in prewashing solution (50 mM Tris-HCl pH7.5, 1 M NaCl, 1 mM EDTA, 0.1% (w/v) sarcosine) at 65° C. for 30 minutes; stripped in 0.4 M sodium hydroxide at 65° C. for 30 minutes; then washed in a solution of 0.2 M Tris-HCl pH 8.0, 0.1×SSC, 0.1% (w/v) SDS at 65° C. for 30 minutes and finally rinsed in 2×SSC, 1.0% (w/v) SDS.

The group A filters of the duplicate lifts from the Kardinal cDNA library were screened with <sup>32</sup>P-labelled fragments of an NcoI fragment from pCGP1805 containing the petunia Ht1 (OGR-38) cDNA clone, while the group B filters were screened with <sup>32</sup>P-labelled fragments of EcoRI/SspI fragment from pCGP246 containing the snapdragon F3'H clone.

Hybridization conditions included a prehybridization step in 10% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42° C. for at least 1 hour. The <sup>32</sup>P-labelled fragment (2×10<sup>6</sup> cpm/ml) was then added to the hybridization solution and hybridization was continued at 42° C. for a further 16 hours. The filters were then washed in 2×SSC, 1% (w/v) SDS for 2 hours followed by 1×SSC, 1% (w/v) SDS for 1 hour and finally in 0.2×SSC/1% (w/v) SDS for 2 hours. The filters were exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

Four strongly-hybridizing plaques (R1, R2, R3, R4) were picked into PSB and rescreened to isolate pure plaques. The

plasmids contained in the λZAP bacteriophage vector were rescued and digested with EcoRI to release the cDNA inserts. Clone R1 contained a 1.0 kb insert while clones R2, R3 and R4 contained inserts of approximately 1.3 kb each. Sequence data were generated from the 3' and 5' ends of the R4 cDNA insert.

The rose R4 putative F3'H sequence was compared with that of the petunia OGR-38 F3'H sequence. At the nucleotide level, the R4 cDNA clone showed 63.2% and 62.1% similarity over 389 nucleotides at the 5' end and 330 nucleotides at the 3' end, respectively. At the amino acid level, the R4 clone showed 65.4% and 73.9% similarity over 130 amino acids at the 5' end and 69 amino acids at the 3' end, respectively. Based on the high sequence similarity of the Rose R4 cDNA clone to that of the petunia F3'H cDNA clone (OGR-38), a corresponding "full-length" cDNA clone was isolated, as described in Example 25, below.

#### EXAMPLE 25

##### Isolation of a Full-length Rose F3'H cDNA

In order to isolate a "full-length" F3'H cDNA clone from Rose, the *Rosa hybrida* cv Kardinal petal cDNA library described in Example 24 was screened with <sup>32</sup>P-labelled fragments of the rose R4 cDNA clone, described above.

A total of 1.9×10<sup>6</sup> pfus of the amplified library were plated onto NZY plates at a density of 100,000 pfus per 15 cm diameter plate after transfecting XL1-Blue MRF' cells, and incubated at 37° C. for 8 hours. After incubation at 4° C. overnight, duplicate lifts were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Screening of Kardinal cDNA Library for Full-length F3'H cDNA Clones

Prior to hybridization, the duplicate plaque lifts were treated as described in Example 24.

The duplicate lifts from the Kardinal cDNA library were screened with <sup>32</sup>P-labelled fragments of an EcoRI fragment from the rose R4 cDNA clone.

Hybridization conditions included a prehybridization step in 50% (v/v) formamide, 1 M NaCl, 10% (w/v) dextran sulphate, 1% (w/v) SDS at 42° C. for at least 1 hour. The <sup>32</sup>P-labelled fragment of the rose R4 cDNA clone (1×10<sup>6</sup> cpm/mL) was then added to the hybridization solution and hybridization was continued at 42° C. for a further 16 hours. The filters were then washed in 2×SSC, 1% (w/v) SDS at 42° C. for 2×1 hour and exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

Seventy-three strongly-hybridizing plaques (1-73) were picked into 1 mL of PSB and stored at 4° C. overnight. 100 μL of each was then aliquoted into a microtitre tray as an ordered array.

XL1-Blue MRF' cells were added to 10 mL of molten NZY top agar, poured onto NZY plates (15 cm diameter) and allowed to set. A replica plating device was used to transfer the 73 phage isolates in an ordered array onto the NZY plate previously inoculated with the XL1-Blue MRF' cells. After incubation at 37° C. for 6 hours followed by 4° C. overnight, triplicate lifts (arrays 1, 2 and 3) were taken onto Colony/Plaque Screen™ filters (DuPont) and treated as recommended by the manufacturer.

Prior to hybridization, the duplicate plaque lifts were treated as described in Example 24.

The 3 arrays were screened with <sup>32</sup>P-labelled fragments of a) an EcoRI/SalI fragment covering the 5' end of the rose R4 cDNA clone, b) an EcoRI/ClaI fragment covering the 5' end of the rose R4 cDNA clone or c) an EcoRI fragment of the

entire rose R4 cDNA clone using the hybridisation and washing conditions described above, except that the final wash was in 0.1×SSC, 0.1% (w/v) SDS at 65° C. for 30 minutes. The filters were exposed to Kodak XAR film with an intensifying screen at -70° C. for 16 hours.

All 73 plaques hybridised with the full R4 cDNA clone (EcoRI fragment) whilst a total of only 17 hybridised with the 5' end of the R4 cDNA clone (either EcoRI/SalI or the EcoRI/ClaI fragments). The 17 phage isolates were rescreened as described above to isolate purified plaques. Pure plaques were Stained from 9 out of the 17 (2, 4, 26, 27, 34, 38, 43, 44, 56). The plasmids contained in the λZAP bacteriophage vector were rescued and the sizes of the cDNA inserts were determined using an EcoRI digestion. The cDNA inserts ranged from 0.9 kb to 1.9 kb. Of the nine, only #34 (named pCGP2158) and #38 (named pCGP2159) contained cDNA inserts of approximately 1.9 kb. Sequence data were generated from the 3' and 5' ends of the cDNA inserts and showed that clones #34 and #38 represented the same gene.

The complete sequence of the rose cDNA clone (#34) contained in the plasmid pCGP2158 was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook et al., 1989). The sequence (SEQ ID NO:14) contained an open reading frame of 1696 bases which encodes a putative polypeptide of 520 amino acids (SEQ ID NO:15).

The nucleotide and predicted amino acid sequences of the rose F3'H #34 cDNA clone (SEQ ID NO:14 and SEQ ID NO:15) were compared with those of the petunia OGR-38 F3'H cDNA clones (SEQ ID NO:1 and SEQ ID NO:2) and the snapdragon sd F3'H clone (SEQ ID NO:5 and SEQ ID NO:6). The rose F3'H #34 cDNA clone showed 64.7% similarity, over 1651 nucleotides and 72.7% similarity, over 509 amino acids, to that of the petunia OGR-38 cDNA clone, and 67.2% similarity, over 1507 nucleotides, and 68.9% similarity, over 502 amino acids, to that of the snapdragon sdF3'H clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)-(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7-11 are in Example 34, at the end of the specification.

#### EXAMPLE 26

##### Stable Expression of the Rose F3'H cDNA Clone (#34) in Petunia Petals—Complementation of a ht1/ht1 Petunia Cultivar

###### Preparation of pCGP2166

Plasmid pCGP2166 (FIG. 16) was constructed by cloning the cDNA insert from pCGP2158 in a "sense" orientation behind the Mac promoter (Comai et al., 1990) of pCGP293 (Brugliera et al., 1994). The plasmid pCGP2158 was digested with EcoRI to release the cDNA insert. The overhanging 5' ends were filled in using DNA polymerase (Klenow fragment) (Sambrook et al., 1989). The cDNA fragment was isolated and ligated with filled in BamHI ends of the pCGP293 binary vector. Correct insertion of the fragment in pCGP2166 was established by restriction enzyme analysis of DNA isolated from gentamycin-resistant transformants.

The binary vector pCGP2166 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9.

The pCGP166/AGL0 cells were then used to transform Skr4×SW63 petunia plants (also described in Example 9), to test for stable expression and activity of the enzyme encoded by the gene corresponding to the rose #34 cDNA clone.

#### EXAMPLE 27

##### Transgenic Plant Phenotype Analysis pCGP2166 in Skr4×SW63

The expression of the introduced rose F3'H cDNA in the Skr4×SW63 hybrid had a marked effect on flower colour. The stamen tissue of the non-transgenic control is white, whereas the same tissue in most of the transgenic plants was pink. In addition, expression of the rose F3'H cDNA in the Skr4×SW63 hybrid conferred a dark pink hue (RHSCC# 64° C. and 74° C.) to the corolla, which is normally pale lilac (RHSCC# 75C). The colour codes are taken from the Royal Horticultural Society's Colour Chart (RHSCC). They provide an alternative means by which to describe the colour phenotypes observed. The designated numbers, however, should be taken only as a guide to the perceived colours and should not be regarded as limiting the possible colours which may be obtained.

Acid-hydrolysed floral extracts (see Example 11) were run in a Forestal solvent system (HOAc:water:HCl; 30:10:3) (Markham, 1982). The 3' hydroxylated flavonoids, peonidin and quercetin, were readily detected in the petal limbs of the transgenic plants. Only kaempferol and a small amount of malvidin were detected in the non-transgenic Skr4×SW63 control.

The accumulation of the 3'-hydroxylated anthocyanidin, peonidin and the flavonol, quercetin, in the petals of the transgenic Skr4×SW63/pCGP2166 plants correlated with the pink and dark pink colours observed in the petals of the same plants.

###### Preparation of pCGP2169

The binary construct pCGP2169 (FIG. 17) was prepared by cloning the cDNA insert from pCGP2158 in a "sense" orientation between the CaMV35S promoter (Franck et al., 1980; Guilley et al., 1982) and ocs terminator (De Greve et al., 1982). The plasmid pCGP1634 contained a CaMV35S promoter, β-glucuronidase (GUS) reporter gene encoded by the *E. coli* uidA locus (Jefferson et al., 1987) and ocs terminator region in a pUC19 vector. The plasmid pCGP2158 was digested with NcoI/XbaI to release the cDNA insert. The plasmid pCGP1634 was also digested with NcoI/XbaI to release the backbone vector containing the CaMV35S promoter and the ocs terminator. The fragments were isolated and ligated together to produce pCGP2167. The plasmid pCGP2167 was subsequently digested with PvuII to release the expression cassette containing the CaMV35S promoter, the rose F3'H cDNA clone and the ocs terminator. This expression cassette fragment was isolated and ligated with SmaI ends of pWTT2132 binary vector (DNA Plant Technology Corporation; Oakland, Calif.) to produce pCGP2169 (FIG. 17).

The binary vector pCGP2169 was introduced into *A. tumefaciens* strain AGL0 cells, as described in Example 9. The pCGP2169/AGL0 cells are used to transform rose plants, to reduce the amount of 3'-hydroxylated flavonoids.

#### EXAMPLE 28

##### Isolation of a Putative F3'H cDNA Clone from Chrysanthemum

In order to isolate a chrysanthemum F3'H cDNA clone, a chrysanthemum cv. Red Minstral petal cDNA library was

screened with <sup>32</sup>P-labelled fragments of the petunia Ht1 cDNA clone (OGR-38), contained in pCGP1805.

Construction of a Petal cDNA Library from Chrysanthemum cv. Red Minstral

Total RNA was prepared from the petals (stages 3 to 5) of chrysanthemum cv. Red Minstral using Trizol™ reagent (Life Technologies) (Chomczynski and Sacchi, 1987) according to the manufacturer's recommendations. Poly(A)<sup>+</sup> RNA was enriched from the total RNA, using a mRNA isolation kit (Pharmacia) which relies on oligo-(dT) affinity spun-column chromatography.

A Superscript™ cDNA synthesis kit (Life Technologies) was used to construct a petal cDNA library in ZipLox using 5 μg of poly(A)<sup>+</sup> RNA isolated from stages 3 to 5 of Red Minstral as template.

30,000 pfus of the library were plated onto LB plates (Sambrook et al., 1989) at a density of 3,000 pfus per 15 cm plate after transfecting Y1090r-, and incubated at 37° C. for 16 hours. After incubation at 4° C. for one hour, duplicate lifts were taken onto Hybond N+™ filters (Amersham) and treated as recommended by the manufacturer.

Screening of the Red Minstral DNA Library

The duplicate lifts from the Red Minstral petal cDNA library were screened with <sup>32</sup>P-labelled fragments of the 1.8 kb Asp718/BamHI insert from pCGP1805.

Hybridization conditions included a prehybridization step in 1 mM EDTA (pH8.0), 0.5MNa<sub>2</sub>HPO<sub>4</sub> (pH7.2), 7% (w/v) SDS (Church and Gilbert, 1984) at 65° C. for at least 1 hour. The <sup>32</sup>P-labelled fragments (1×10<sup>6</sup> cpm/mL) were then added to the hybridization solution and hybridization was continued at 65° C. for a further 16 hours. The filters were then washed in 2×SSC, 0.1% (w/v) SDS at 65° C. for 2×1 hour and exposed to Kodak BioMax™ film with an intensifying screen at -70° C. for 48 hours.

Eight strongly-hybridizing plaques were picked into PSB (Sambrook et al., 1989). Of these, 2 (RM6i and RM6ii) were rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. The plasmids contained in the λZipLox bacteriophage vector were rescued according to the manufacturer's protocol and sequence data was generated from the 3' and 5' ends of the cDNA inserts. The partial sequences of the RM6i and RM6ii cDNA inserts were compared with the complete sequence of the petunia OGR-38 F3'H cDNA clone. The RM6i cDNA clone showed relatively high sequence similarity with that of the petunia OGR-38 cDNA clone, and was further characterised.

The RM6i cDNA insert contained in pCHRM1 was released upon digestion with EcoRI and was approximately 1.68 kb. The complete sequence of RM6i cDNA clone (SEQ ID NO:16) contained in the plasmid pCHRM1 was determined by compilation of sequence from subclones of the RM6i cDNA insert.

The nucleotide and predicted amino acid sequences of the chrysanthemum RM6i cDNA insert (SEQ ID NO:16 and SEQ ID NO:17) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequence of the chrysanthemum RM6i cDNA insert showed 68.5% similarity, over 1532 nucleotides, and 73.6% similarity, over 511 amino acids, to that of the petunia OGR-38 F3'H<sub>1</sub> cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)-(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7-11 are in Example 34, at the end of the specification.

Conduction of pLN85 (Antisense Binary)

A plasmid designated pLN84 was constructed by cloning the RM6i cDNA insert from pCHRM1 in the "antisense" orientation behind the complete CaMV35S promoter contained in pART7 (Gleave 1992). The plasmid pCHRM1 was digested with NotI to release the cDNA insert. The RM6i cDNA fragment was blunt-ended using T4 DNA polymerase (Sambrook et al., 1989) and purified, following agarose gel electrophoresis and GELase (Epicentre Technologies). The purified fragment was ligated with SmaI ends of the pART7 shuttle vector to produce pLN84. The plasmid pLN84 was subsequently digested with NotI to release the expression cassette containing CaMV35S: RM6i cDNA: ocs. The expression cassette was isolated as a single fragment and ligated with NotI ends of the pART727 binary vector (Gleave, 1992) to produce pLN85 (FIG. 18). Correct insertion of the fragment was established by restriction enzyme analysis of DNA isolated from streptomycin-resistant *E. coli* transformants.

The binary vector pLN85 is introduced into chrysanthemum plants via Agrobacterium-mediated transformation, as described in Ledger et al, 1991), to reduce the amount of 3'-hydroxylated flavonoids.

#### EXAMPLE 29

##### Isolation of a Putative F3'H cDNA Clone from *Torenia fournieri*

In order to isolate a torenia F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a *Torenia fournieri* cv. Summer Wave petal cDNA library, under low stringency conditions.

Construction of *Torenia fournieri* cv. Summer Wave Petal cDNA Library

A directional petal cDNA library was prepared from Summer Wave flowers, essentially as described in Example 4.

Screening of Summer Wave Petal cDNA Library

Lifts of a total of 200,000 of the amplified Summer Wave petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5×SSC, 1% (w/v) SDS at 37° C. for 16 hours. The filters were then washed in 5×SSC, 1% (w/v) SDS at 65° C. for 1 hour.

The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Twelve strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. Most of the twelve clones contained cDNA inserts of approximately 1.8 kb. One clone, THT52, contained the longest 5' non-coding-region sequence. The complete sequence of the torenia cDNA clone (THT52), contained in the plasmid pTHT52, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook et al., 1989). The sequence (SEQ ID NO:18) contained an open reading frame of 1524 bases which encodes a putative polypeptide of 508 amino acids (SEQ ID NO:19).

The nucleotide and predicted amino acid sequences of the torenia TH<sub>1</sub>T<sub>2</sub> cDNA clone (SEQ ID NO:18 and SEQ ID

NO:19) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The torenia THT52 cDNA clone showed 63.6% similarity, over 1694 nucleotides, and 67.4% similarity, over 515 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in Table 7 and in Tables 8, 9, 10, 11 and 12, respectively. These Tables are in Example 34, at the end of the specification.

#### EXAMPLE 30

##### The 3'H Assay of the Torenia THT cDNA Clone Expressed in Yeast Construction of pYTHT6

The plasmid pYTHT6 (FIG. 19) was constructed by cloning the cDNA insert from pTHT6 in a "sense" orientation behind the yeast glyceraldehyde-3-phosphate dehydrogenase promoter of pYE22m (Tanaka et al., 1988). The plasmid pTHT6 contained the THT6 cDNA clone. THT6 is identical to THT52, except that its 5' non-coding region is 75 bp shorter.

The 1.7 kb THT6 cDNA insert was released from the plasmid pTHT6 upon digestion with EcoI/XhoI. The THT6 cDNA fragment was isolated, purified and ligated with EcoRI/SalI ends of pYE22m to produce pYTHT6.

Yeast transformation, preparation of yeast extracts and the F3'H assay are described in Example 6.

F3'H activity was detected in extracts of G1315/pYTHT6, but not in extracts of non-transgenic yeast. From this it was concluded that the THT6 cDNA insert contained in pYTHT6, encoded a F3'H.

#### EXAMPLE 31

##### Isolation of a Putative F3'H cDNA Clone from *Pharbitis nil* (Japanese Morning Glory)

In order to isolate a morning glory F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a Japanese morning glory petal cDNA library, under low stringency conditions.

Construction of Japanese Morning Glory Petal cDNA Library

The petal cDNA library from young petals of *Pharbitis nil* (Japanese morning glory) was obtained from Dr Iida (National Institute of Basic Biology, Japan).

Screening of Japanese Morning Glory Petal cDNA Library

Lifts of a total of 200,000 of the amplified Japanese morning glory petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5×SSC, 1% (w/v) SDS at 37° C. for 16 hours. The filters were then washed in 5×SSC, 1% (w/v) SDS at 65° C. for 1 hour. The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Twenty strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. One clone (MHT85) contained a 1.8 kb insert. The complete

sequence of the Japanese morning glory cDNA clone (MHT85) (SEQ ID NO:20), contained in the plasmid pMHT85, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook et al., 1989). The MHT85 sequence appears to be 5 bases short of "full-length".

The nucleotide and predicted amino acid sequences of the Japanese morning glory MHT85 cDNA clone (SEQ ID NO:20 and SEQ ID NO:21) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The Japanese morning glory MHT85 cDNA clone showed 69.6% similarity, over 869 nucleotides, and 74.8% similarity, over 515 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)-(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7-11 are in Example 34, at the end of the specification.

#### EXAMPLE 32

##### Isolation of a Putative F3'H cDNA Clone from *Gentiana triflora*

In order to isolate a gentian F3'H cDNA clone, the petunia Ht1-link F3'H cDNA clone (OGR-38), contained in pCGP1805, was used to screen a *Gentiana triflora* Pall. var japonica Hara petal cDNA library, under low stringency conditions.

Construction of Gentian Petal cDNA Library

A petal cDNA library was prepared from *Gentiana triflora* Pall. var japonica Hara flowers, as described by Tanaka et al., 1996.

Screening of Gentian Petal cDNA Library

Lifts of a total of 200,000 of the amplified gentian petal cDNA library were screened with DIG-labelled fragments of the 1.8 kb OGR-38 cDNA insert from pCGP1805. A DIG DNA labelling and detection kit from Boehringer-Mannheim was used according to the manufacturer's recommendations.

Hybridizations were carried out in 30% (v/v) formamide, 5×SSC, 1% (w/v) SDS at 37° C. for 16 hours. The filters were then washed in 5×SSC, 1% (w/v) SDS at 65° C. for 1 hour. The signals were visualized following the protocol of the DIG DNA labelling and detection kit.

Fifteen strongly-hybridizing plaques were picked into PSB and rescreened to isolate pure plaques. The plasmids contained in the λZAPII bacteriophage vector were rescued and digested with EcoRI/XhoI to release the cDNA inserts. One clone (GHT13) contained a 1.8 kb insert. The sequence of the partial gentian cDNA clone (GHT13) (SEQ ID NO:22), contained in the plasmid pGHT13, was determined by compilation of sequence from different pUC18 subclones obtained using standard procedures for the generation of randomly-overlapping clones (Sambrook et al 1989).

The nucleotide and predicted amino acid sequences of the gentian GHT13 cDNA clone (SEQ ID NO:22 and SEQ ID NO:23) were compared with those of the petunia OGR-38 F3'H cDNA clone. The gentian GHT13 cDNA clone showed 68.3% similarity, over 1519 nucleotides, and 71.8% similarity, over 475 amino acids, to that of the petunia OGR-38 cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all

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of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)–(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7–11 are in Example 34, at the end of the specification.

## EXAMPLE 33

## Isolation of Putative F3'H cDNA Clone from Lisianthus

In order to isolate a lisianthus F3'H cDNA clone, the petunia Ht1-linked F3'H cDNA clone EA (OGR-38), contained in pCGP1805, was used to screen a lisianthus petal cDNA library, under low stringency conditions.

## Construction and Screening of Lisianthus Petal cDNA Library

10,000 pfus of a lisianthus petal cDNA library described by Davies et al. (1993) and Markham and Offman (1993) were plated onto LB plates (Sambrook et al., 1989) at a density of 3,000 pfus per 15 cm plate after transfecting Y1090r-, and incubated at 37° C. for 16 hours. After incubation at 4° C. for one hour, duplicate lifts were taken onto Hybond N+™ filters (Amersham) and treated as recommended by the manufacturer.

The duplicate lifts from the lisianthus line #54 petal cDNA library were screened with <sup>32</sup>P-labelled fragments of the 1.8 kb AsP718/BAmHI insert from pCGP1805.

Hybridization conditions included a prehybridization step in 1 mM EDTA (pH8.0), 0.5MNa<sub>2</sub>HPO<sub>4</sub> (pH7.2), 7% (w/v) SDS (Church and Gilbert, 1984) at 55° C. for at least 1 hour. The <sup>32</sup>P-labelled fragments (1×10<sup>6</sup> cpm/mL) were then added to the hybridization solution and hybridization was continued at 55° C. for a further 16 hours. The filters were then washed in 2×SSC, 0.1% (w/v) SDS at 55° C. for 2×15 minutes, and exposed to Kodak BioMax™ film with an intensifying screen at -70° C. for 18 hours.

Twelve strongly-hybridizing plaques were picked into PSB (Sambrook et al., 1989) and rescreened to isolate purified plaques, using the hybridization conditions as described for the initial screening of the cDNA library. Sequence data were generated from the 3' and 5' ends of the cDNA inserts of four clones.

Based on sequence comparisons, pL3-6 showed similarity with the petunia OGR-38 F3'H cDNA clone and was further characterised.

The 2.2 kb cDNA insert, contained in pL3-6, was subsequently found to contain 3 truncated cDNA clones, the longest (L3-6) having high sequence similarity to the petunia OGR-38 cDNA sequence. The sequence of this L3-6 partial cDNA clone contained in the plasmid pL3-6 was determined by compilation of sequence from subclones of the L3-6 cDNA insert (SEQ ID NO:24).

The nucleotide and predicted amino acid sequences of the lisianthus L3-6 cDNA clone (SEQ ID NO:24 and SEQ ID NO:25) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2). The sequence of the lisianthus L3-6 cDNA clone showed 71.4% similarity, over 1087 nucleotides, and 74.6% similarity, over 362 amino acids, to that of the petunia OGR-38 F3'H cDNA clone.

An alignment of the petunia, carnation, snapdragon, arabidopsis, rose, chrysanthemum and torenia sequences, all

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of which are disclosed in this specification, and various summaries of comparisons of sequence similarities among the nucleotide and corresponding amino acid sequences, can be found in FIGS. 20(i)–(v) and in Tables 7, 8, 9, 10, and 11 respectively. Tables 7–11 are in Example 34, at the end of the specification.

Further investigation of the remaining clones isolated from the screening of the lisianthus library identified another putative F3'H cDNA clone (L3-10), contained in the plasmid pL3-10. The L3-10 cDNA insert is approximately 1.8 kb and appears to represent a “full-length” clone.

## EXAMPLE 34

## Alignments and Comparisons Among Nucleotide and Amino Acid Sequences Disclosed Herein

Multiple sequence alignments were performed using the ClustalW program as described in Example 3. FIG. 20(i)–(v) provide a multiple sequence alignment of the predicted amino acid sequences of petunia OGR-38 (A) (SEQ ID NO:2); carnation (B) (SEQ ID NO:4); snapdragon (C) (SEQ ID NO:6); arabidopsis Tt7 coding region (D) (SEQ ID NO:42); rose (E) (SEQ ID NO:15) chrysanthemum (F) (SEQ ID NO:17); torenia (G) (SEQ ID NO:19); morning glory (H) (SEQ ID NO:21); gentian (partial sequence) (I) (SEQ ID NO:23); lisianthus (partial sequence) (J) (SEQ ID NO:25) and the petunia 651 cDNA (K) (SEQ ID NO:41). Conserved amino acids are shown in bolded capital letters and are boxed and shaded. Similar amino acids are shown in capital letters and are only lightly shaded, and dissimilar amino acids are shown in lower case letters.

The nucleotide and predicted amino acid sequences of the rose F3'H; #34 cDNA clone (SEQ ID NO:14 and SEQ ID NO:15) were compared with those of the petunia OGR-38 F3'H cDNA clone (SEQ ID NO:1 and SEQ ID NO:2) and the snapdragon sd F3'H clone (SEQ ID NO:5 and SEQ ID NO:6). The rose F3'H #34 cDNA clone showed 64.7% similarity, over 1651 nucleotides and 72.7% similarity, over 509 amino acids, to that of the petunia OGR-38 cDNA clone, and 67.2% similarity, over 1507 nucleotides, and 68.9% similarity, over 502 amino acids, to that of the snapdragon sdF3'H clone.

Nucleotide and amino acid sequences of the F3'H cDNA clones from the above mentioned species and the coding region of the genomic clone from arabidopsis were compared using the LFASTA program, as described in Example 3. Summaries of similarity comparisons are presented in Tables 8 to 12, below.

TABLE 7

Percentage of sequence similarity between F3'H sequence of petunia OGR-38 and F3'H sequences from other species and other P450 molecules				
Species/Clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to OGR-38/ no. nt (area of similarity)	% similarity to OGR-38/ no. aa (area of similarity)
Petunia OGR-38	1789nt	512aa		
Snapdragon	11711nt	512aa	69.0%/1573nt (19-1578)	72.2%/507aa (1-504)
F3'H cDNA				
Arabidopsis partial	971nt	270aa	64.7%/745nt (854-1853)	63.7%/248aa (269-510)
F3'H cDNA				
Arabidopsis Tt7 coding region	1774nt	513aa	65.4%/1066nt	67.1%/511aa
Carnation	1745nt	496aa	67.3%/1555nt (28-1571)	71.5%/488aa (17-503)
F3'H cDNA				
Rose	1748nt	513aa	64.7%/165nt (56-1699)	72.7%/509aa (7-510)
F3'H cDNA				
Gentian	1667nt	476aa	68.3%/1519nt (170-1673)	71.8%/475aa (40-510)
partial F3'H cDNA				
Morning Glory	1824nt	517aa	69.6%/869nt (60-1000)	74.8%/515aa (3-503)
F3'H cDNA				
Chrysanthemum	1660nt	508aa	68.5%/1532nt (50-1580)	73.6%/511aa (1-510)
F3'H cDNA				
Lisianthus	1214nt	363aa	71.4%/1087nt (520-1590)	74.6%/362aa (160-510)
partial F3'H cDNA				
Torenia	1815nt	508aa	63.6%/1694nt (90-1780)	67.4%/515aa (1-510)
F3'H cDNA				
Petunia Hf1	1812nt	508aa	58.9%/1471nt (29-1474)	49.9%/513aa (1-511)
cDNA				
Petunia Hf2	1741nt	508aa	58.9%/1481nt (37-1498)	49.1%/511aa (3-510)
cDNA				
Petunia 651	1716nt	496aa	53.5%/1284nt (50-1309)	38.0%/502aa (7-503)
cDNA				
Mung Bean	1766nt	505aa	56.0%/725nt (703-1406)	29.2%/511aa (1-503)
C4H cDNA				

TABLE 8

Percentage of sequence similarity between F3'H sequence of Snapdragon and F3'H sequences from other species and other P450 molecules				
Species/Clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to snapdragon/no. nt	% similarity to snapdragon/no. aa
Snapdragon	1711nt	512aa		
Petunia OGR-38	1789nt	512aa	69.0%/1573nt	72.2%/507aa
F3'H cDNA				
Arabidopsis	971nt	270aa	64.5%/740nt	60.4%/240aa
partial F3'H cDNA				
Carnation	1745nt	496aa	66.7%/1455nt	68.4%/487aa
F3'H cDNA				
Torenia	1815nt	508aa	67.6%/1603nt	70.3%/505aa
F3'H cDNA				
Rose	1748nt	513aa	67.2%/1507nt	68.9%/502aa
F3'H cDNA				
Petunia Hf1	1812nt	508aa	57.3%/1563nt	49.3%/491aa
cDNA				
Petunia Hf2	1741nt	508aa	57.7%/1488nt	47.8%/508aa
cDNA				
Petunia 651	1716nt	496aa	54.4%/1527nt	39.0%/493aa
cDNA				
Mung Bean	1766nt	505aa	50.6%/1344nt	32.0%/490aa
C4H cDNA				

TABLE 9

Percentage of sequence similarity between F3'H sequence of Arabidopsis and F3'H sequences from other species and other P450 molecules				
Species/Clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to Arabidopsis/no. nt	% similarity to Arabidopsis/no. aa
Arabidopsis	971nt	270aa		
Petunia OGR-38 F3'H cDNA	1789nt	512aa	64.7%/745nt	63.7%/245%
Snapdragon F3'H CDNA	1711nt	512aa	64.5%/740nt	60.4%/240aa
Carnation F3'H cDNA	1745nt	496aa	64.7%/782nt	60.6%/241aa
Rose F3'H cDNA	1748nt	513aa	68.5%/739nt	63.7%/248aa
Petunia 651 cDNA	1716nt	496aa	57.0%/521nt	40.5%/227aa
Petunia Hf1 cDNA	1812nt	508aa	58.2%/632nt	46.5%/243aa
Petunia Hf2 cDNA	1741nt	508aa	57.4%/632nt	46.1%/243aa

TABLE 10

Percentage of sequence similarity between F3'H sequence of Rose and F3'H sequences from other species and other P450 molecules				
Species/clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to Rose/no. nt	% similarity to Rose/no. aa
Rose	1748bp	513aa		
Petunia OGR-38 Fe'H cDNA	1789bp	5123aa	64.7%/1651nt	72.7%/509aa
Snapdragon Fe'H cDNA	1711bp	512aa	67.2%/1507	68.9%/502aa
Carnation Fe'H cDNA	1745bp	496aa	67.4%/1517nt	72.6%/486aa
Arabidopsis partial F3'H cDNA	971bp	270aa	68.5%/739nt	63.7%/248aa
Petunia 651 cDNA	1716bp	496aa	53.1%/1182nt	37.8%/502aa
Petunia Hf1 cDNA	1812bp	506aa	57%/1366nt	49.9%/503aa
Petunia Hf2 cDNA	1741bp	508aa	57.3%/1331nt	49.1%/505aa
Mung Bean C4H cDNA	1766bp	505aa	52.4%/1502nt	32.0%/510aa

TABLE 11

Percentage of sequence similarity between coding region of Arabidopsis tt7 genomic sequence and F3'H cDNA sequences from other species and other P450 molecules				
Species/Clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to Arabidopsis tt7/no. nt	% similarity to Arabidopsis tt7/no. aa
Arabidopsis Tt7 coding region	1774nt	513aa		
Petunia OGR-38 F3'H cDNA	1789nt	512aa	65.4%/1066nt	67.1%/511aa
Snapdragon F3'H cDNA	1711nt	512aa	62.7%/990nt	64.9%/504aa
Carnation F3'H cDNA	1745nt	496aa	63.2%/1050nt	65.9%/495aa
Rose F3'H cDNA	1748nt	513aa	65.5%/1076nt	68%/512aa

TABLE 11-continued

Percentage of sequence similarity between coding region of Arabidopsis tt7 genomic sequence and F3'H cDNA sequences from other species and other P450 molecules				
Species/Clone	Number of nucleotides (nt)	Number of amino acids (aa)	% similarity to Arabidopsis tt7/no. nt	% similarity to Arabidopsis tt7/no. aa
Petunia 651 cDNA	1716nt	496aa	56.5%/990nt	36.5%/502aa
Petunia Hf1 F3'H cDNA	1812nt	506aa	56.8%/995nt	47.5%/509aa
Petunia Hf2 F3'H cDNA	1741nt	508aa	55.2%/1063nt	46.8%/509aa

Those skilled in the art, will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

## REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. *J. Mol. Biol.* 215: 403410, 1990.
- Ashikari, T., Kiuchi-Goto, N., Tanaka, Y., Shibano, Y., Amachi, T., and Yoshizumi, H. *Appl. Microbiol. Biotechnol.* 30: 515-520, 1989.
- Baird, W. V. and Meagher, R. B. *EMBO J.* 6, 3223-3231, 1987.
- Bechtold, N., Ellis, J. and Pelletier, G. C. R. Acad. Sci. Paris, Sciences de la vie 316: 1194-1199, 1993.
- Bethesda Research Laboratories. BRL pUC host: *E. coli* DH5 $\alpha$ <sup>TM</sup> competent cells. *Bethesda Res. Lab. Focus.* 8(2): 9, 1986.
- Brugliera, F., Holton, T. A., Stevenson, T. W., Farcy, E., Lu, C-Y and Cornish, E. C. *Plan J.* 5(1): 81-92, 1994.
- Church, G. M. and Gilbert, W. *PNAS USA*, 81: 1991-1995.



- Chomczynski, P. and Sacchi, N. *Anal Biochem.* 162: 156–159.
- Comai, L., Moran, P. and Maslyar, D., *Plant Mol. Biol.* 15: 373–381, 1990.
- Cornu, A., Farcy, E., Maizonnier, D., Haring, M., Veerman, W. and Gerats, A. G. M., In: *Genetic maps—Locus maps of complex genomes*. 5th edition, Stephen J. O'Brien (ed.), Cold Spring Harbor Laboratory Press, USA, 1990.
- Davies et al., *Plant Science*, 95: 67–77, 1993.
- D'Alessio et al., *Focus*, 14: 76–79, 1992
- De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M and Schell, J. *J. Mol Appl Genet.* 1: 499–511.
- Dellaporta, S. J., Wood, J. and Hick, J. B., *Plant Mol. Biol. Rep.* 1: 19–21, 1983.
- Del Sal, G., Manfioletti, G. and Schneider, C. *Biotechniques*, 7(S): 514–519, 1989.
- Doodeman, M., Gerats, A. G. M., Schram, A. W., De Vlaming, P. and Bianchi, F., *Theor. Appl. Genet.* 67: 357–366, 1984.
- Dooner, H. K., Robbins, T. R. and Jorgensen, R. A. *Ann. Rev. Genet.* 25: 173–199, 1991.
- Ebel, J. and Hahlbrock, K., In: *The Flavonoids: Advances in Research Since 1980*. Harbounie, J. B. (ed.), Academic Press, New York, USA, 641679, 1988.
- Forkmann, G. and Stotz, G. *Z. Naturforsch.* 36c:411–416, 1981.
- Forkmann, G. *Plant Breeding* 106: 1–26, 1991.
- Franck, A., Guilley, H., Jonard, G. Richards, K. and Hirth, L. *Cell*, 21, 285–294, 1980.
- Frohman, M. A., Dush, M. K., Martin, G. R. *Proc. Natl. Acad. Sci. USA* 85: 8998–9002, 1988.
- Gamborg, O. L., Miller, R. A. and Ojira, K., *Exp. Cell Res.* 50: 151–158, 1968.
- Garfinkel, D. J. and Nester, E. W. *J. Bacteriol.* 144:732–743, 1980.
- Gleave, A. P. *Plant Molecular Biology* 20: 1203–1207, 1992.
- Guilley, H., Dudley, R. K., Jonard, G., Balazs, E. and Richards, K. E. *Cell*, 30, 763–773, 1982.
- Hahlbrock, K. and Grisebach, H., *Annu. Rev. Plant Physiol.* 30: 105–130, 1979.
- Hanahan, D., *J. Mol. Biol.* 166: 557, 1983.
- Haughn, G. W. and Somerville, C. *Molecular and General Genetics* 204: 430–434, 1986.
- Holton, T. A., Brugliera, F. Lester, D. R., Tanaka, Y., Hyland, C. D., Menting, J. G. T., Lu, C., Farcy, E., Stevenson, T. W. and Cornish, E. C., *Nature*, 366, 276–279, 1993.
- Holton, T. A. and Cornish, E. C. *Plant Cell*, 7: 1071–1083, 1995.
- Inoue, H., Nojima, H. and Okayama, H. *Gene*, 96: 23–28, 1990.
- Ito, H., Fukuda, Y., Murata, K. and Kimura, A. *J. Bacteriol.* 153: 163–168, 1983.
- Jefferson, R. A. *Plant Mol. Biol. Rep.* 5: 387–405, 1987.
- Jefferson, R. A., Kavanagh, T. A., and Bevan, M. W. *EMBO J.* 6: 3901–3907, 1987.
- Koorneef, M, Luiten, W., de Vlaming, P. and Schram, A. *W. Arabidopsis Information Service* 19: 113–115, 1982.
- Kozak, M. J. *Cell. Biol.* 108: 229, 1989.
- Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Day, M. J., Lincoln, S. E. and Newberg, L. *Genomics*, 121, 185–199, 1987.

- Lazo, G. R., Pascal, A. S. and Ludwig, R. A., *Bio/technology*, 9: 963–967, 1991.
- Ledger, S. E., Delores, S. C. and Given, N. K. *Plant Cell Reports*, 10: 195–199, 1991.
- Liang, P. and Pardee, A. B. *Science*, 257: 967–971, 1992.
- Liang, P., Averboukh, L. and Pardee, A. B. *Nucl. Acids Res.* 21: 3269–3275, 1993.
- Marchuk, D., Drumm, M., Saulino, A., Collins, F. S. *Nucl. Acids Res.* 19: 1154, 1990.
- Markham, K. R., *Techniques of flavonoid identification*, London: Academic Press, 1982.
- Markham, K. R and Offman, D. J. *Phytochem.*, 34: 679–685.
- Martin, C. and Gerats, T. In: *The molecular biology of flowering*. (Jordan, B. R. ed), UK, CAB International, 219–255, 1993.
- McLean, M., Gerats, A. G. M., Baird, W. V. and Meagher, R. B. *J. Heredity* 81: 341–346, 1990.
- Merrifield, J. *Am. Chem. Soc.* 85: 2149, 1964.
- Mizutani, M., Ward, E., DiMaio, J., Ohta, D., Ryals, J. and Sato, R. *Biochem. Biophys. Res. Commun.* 190: 875–880, 1993.
- Murashige, T. and Skoog, F., *Physiol. Plant*, 15: 73–97, 1962.
- Newman, T., de Bruijn, F. J., Green, P., Keegstra, K., Kende, H., McIntosh, L., Ohlrogge, J., Raikhel, N., Somerville, S., Thomashow, M. *Plant Physiol.* 106: 1241–1255, 1994.
- Pearson, W. R. and Lipman, D. J., *Proc. Natl. Acad. Sci. USA* 85: 2444–2448, 1988.
- Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual* (2nd edition). Cold Spring Harbor Laboratory Press, USA, 1989.
- Schenk, R. U. and Hilderbrandt, A. C., *Can. J. Bot.* 50: 199–204, 1972.
- Schram, A. W., Jonsson, L. M. V. and Bennink, G. J. H., Biochemistry of flavonoid synthesis in *Petunia hybrida*. In: *Petunia Sink*, K. C. (ed.), Springer-Verlag, Berlin, Germany, pp 68–75, 1984.
- Stafford, H. A., *Flavonoid Metabolism*. CRC Press, Inc. Boca Raton, Fla., USA, 1990.
- Stotz, G. and Forkmann, G. *Z. Naturforsch* 37c: 19–23, 1982.
- Tabak, A. J. H., Meyer, H. and Bennink, G. J. H., *Planta* 139, 67–71, 1978.
- Tanaka, Y., Ashikari, T., Shibano, Y., Amachi, T., Yoshizumi, H. and Maltsubara, H. *J. Biochem.* 103: 954–961, 1988.
- Tanaka, Y., Yonekura, K., Fukuchi-Mizutani, M., Fukui, Y., Fujiwara, H., Ashikari, T. and Kusumi, T. *Plant Cell Physiol.* 37(5): 711–716, 1996.
- Turpen, T. H. and Griffith, O. M. *Biotechniques*, 4: 11–15, 1986.
- van Tunen A. J. and Mol J. N. M. In: *Plant Biotechnology* (Grierson, D. ed.) Glasgow: Blackie, 2: 9–31, 1990.
- Wiering, H. and de Vlaming, P., Inheritance and Biochemistry of Pigments. In: *Petunia Sink*, K. C. (ed.), Springer-Verlag, Berlin, Germany, pp 49–65, 1984.
- Wallroth, M., Gerats, A. G. M., Rogers, S. G., Fraley, R. T. and Horsch, R. B., *Mol. Gen. Genet.* 202: 6–15, 1986.

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Leu Ser Leu Ile Leu Tyr Thr Val Ile Phe Ser Phe Leu Leu Gln Phe
   5                               10                               15

att ctt aga tca ttt ttc cgt aaa cgt tac cct tta cca tta cca cca      154
Ile Leu Arg Ser Phe Phe Arg Lys Arg Tyr Pro Leu Pro Leu Pro Pro
  20                               25                               30                               35

ggt cca aaa cca tgg cca att ata gga aac cta gtc cat ctt gga ccc      202
Gly Pro Lys Pro Trp Pro Ile Ile Gly Asn Leu Val His Leu Gly Pro
                               40                               45                               50

aaa cca cat caa tca act gca gcc atg gct caa act tat gga cca ctc      250
Lys Pro His Gln Ser Thr Ala Ala Met Ala Gln Thr Tyr Gly Pro Leu
                               55                               60                               65

atg tat ctt aag atg ggg ttc gta gac gtg gtg gtt gca gcc tcg gca      298
Met Tyr Leu Lys Met Gly Phe Val Asp Val Val Val Ala Ala Ser Ala
   70                               75                               80

tcg gtt gca gct cag ttc ttg aaa act cat gat gct aat ttc tcg agc      346
Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn Phe Ser Ser
   85                               90                               95

cgt cca cca aat tct ggt gca gaa cat atg gct tat aat tat cag gat      394
Arg Pro Pro Asn Ser Gly Ala Glu His Met Ala Tyr Asn Tyr Gln Asp
 100                               105                               110                               115

ctt gtt ttt gca cct tat gga cct aga tgg cgt atg ctt agg aaa att      442
Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile
                               120                               125                               130

tgc tca gtt cac ctt ttc tct acc aag gct tta gat gac ttc cgc cat      490
Cys Ser Val His Leu Phe Ser Thr Lys Ala Leu Asp Asp Phe Arg His
                               135                               140                               145

gtc cgc cag gat gaa gtg aaa aca ctg acg cgc gca cta gca agt gca      538
Val Arg Gln Asp Glu Val Lys Thr Leu Thr Arg Ala Leu Ala Ser Ala
                               150                               155                               160

ggc caa aag cca gtc aaa tta ggt cag tta ttg aac gtg tgc acg acg      586
Gly Gln Lys Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr Thr
 165                               170                               175

aac gca ctc gcg cga gta atg cta ggt aag cga gta ttt gcc gac gga      634
Asn Ala Leu Ala Arg Val Met Leu Gly Lys Arg Val Phe Ala Asp Gly
 180                               185                               190                               195

agt ggc gat gtt gat cca caa gcg gcg gag ttc aag tca atg gtg gtg      682
Ser Gly Asp Val Asp Pro Gln Ala Ala Glu Phe Lys Ser Met Val Val
                               200                               205                               210

gaa atg atg gta gtc gcc ggt gtt ttt aac att ggt gat ttt att ccg      730
Glu Met Met Val Val Ala Gly Val Phe Asn Ile Gly Asp Phe Ile Pro
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caa ctt aat tgg tta gat att caa ggt gta gcc gct aaa atg aag aag      778
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245 250 255	
aag ggt aaa att ttt gga gaa atg aaa gat ttg ttg agt act ttg atc	874
Lys Gly Lys Ile Phe Gly Glu Met Lys Asp Leu Leu Ser Thr Leu Ile	
260 265 270 275	
tct ctt aaa aat gat gat gcg gat aat gat gga ggg aaa ctc act gat	922
Ser Leu Lys Asn Asp Asp Ala Asp Asn Asp Gly Gly Lys Leu Thr Asp	
280 285 290	
aca gaa att aaa gca tta ctt ttg aac ttg ttt gta gct gga aca gac	970
Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Val Ala Gly Thr Asp	
295 300 305	
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Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg Asn	
310 315 320	
cca aaa ata cta gcc caa gcc cag caa gag atc gac aaa gtc gtt gga	1066
Pro Lys Ile Leu Ala Gln Ala Gln Gln Glu Ile Asp Lys Val Val Gly	
325 330 335	
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gaa gct ata gtc aag gaa acc ttt cgg ctt cat cca tca acc cct ctt	1162
Glu Ala Ile Val Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu	
360 365 370	
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Ser Leu Pro Arg Ile Ala Ser Glu Ser Cys Glu Ile Asn Gly Tyr Phe	
375 380 385	
att cca aaa ggc tca acg ctt ctc ctt aat gtt tgg gcc att gct cgt	1258
Ile Pro Lys Gly Ser Thr Leu Leu Leu Asn Val Trp Ala Ile Ala Arg	
390 395 400	
gat cca aat gca tgg gct gat cca ttg gag ttt agg cct gaa agg ttt	1306
Asp Pro Asn Ala Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe	
405 410 415	
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Leu Pro Gly Gly Glu Lys Pro Lys Val Asp Val Arg Gly Asn Asp Phe	
420 425 430 435	
gaa gtc ata cca ttt gga gct gga cgt agg att tgt gct gga atg aat	1402
Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Asn	
440 445 450	
ttg ggt ata cgt atg gtc cag ttg atg att gca act tta ata cat gca	1450
Leu Gly Ile Arg Met Val Gln Leu Met Ile Ala Thr Leu Ile His Ala	
455 460 465	
ttt aac tgg gat ttg gtc agt gga caa ttg ccg gag atg ttg aat atg	1498
Phe Asn Trp Asp Leu Val Ser Gly Gln Leu Pro Glu Met Leu Asn Met	
470 475 480	
gaa gaa gca tat ggg ctg acc tta caa cgg gct gat cca ttg gtt gtg	1546
Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro Leu Val Val	
485 490 495	
cac cca agg cct cgc tta gaa gcc caa gcg tac att ggg tga	1588
His Pro Arg Pro Arg Leu Glu Ala Gln Ala Tyr Ile Gly	
500 505 510	
gcagcaacag cccatggaga taacatgagt gttaaatgta tgagtctcca tatcttgttt	1648
agtttgttta tgctttggat ttagtagttt ttatattgat agatcaatgt ttgcattgtc	1708
agtaagaata tccgttgctt gtttcattaa ctccaggtgg acaataaaag aagtaatttg	1768
tatgaaaaaa a	1789

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 512

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&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Petunia x hybrida

&lt;400&gt; SEQUENCE: 2

Met Glu Ile Leu Ser Leu Ile Leu Tyr Thr Val Ile Phe Ser Phe Leu  
1 5 10 15  
Leu Gln Phe Ile Leu Arg Ser Phe Phe Arg Lys Arg Tyr Pro Leu Pro  
20 25 30  
Leu Pro Pro Gly Pro Lys Pro Trp Pro Ile Ile Gly Asn Leu Val His  
35 40 45  
Leu Gly Pro Lys Pro His Gln Ser Thr Ala Ala Met Ala Gln Thr Tyr  
50 55 60  
Gly Pro Leu Met Tyr Leu Lys Met Gly Phe Val Asp Val Val Val Ala  
65 70 75 80  
Ala Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn  
85 90 95  
Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Glu His Met Ala Tyr Asn  
100 105 110  
Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu  
115 120 125  
Arg Lys Ile Cys Ser Val His Leu Phe Ser Thr Lys Ala Leu Asp Asp  
130 135 140  
Phe Arg His Val Arg Gln Asp Glu Val Lys Thr Leu Thr Arg Ala Leu  
145 150 155 160  
Ala Ser Ala Gly Gln Lys Pro Val Lys Leu Gly Gln Leu Leu Asn Val  
165 170 175  
Cys Thr Thr Asn Ala Leu Ala Arg Val Met Leu Gly Lys Arg Val Phe  
180 185 190  
Ala Asp Gly Ser Gly Asp Val Asp Pro Gln Ala Ala Glu Phe Lys Ser  
195 200 205  
Met Val Val Glu Met Met Val Val Ala Gly Val Phe Asn Ile Gly Asp  
210 215 220  
Phe Ile Pro Gln Leu Asn Trp Leu Asp Ile Gln Gly Val Ala Ala Lys  
225 230 235 240  
Met Lys Lys Leu His Ala Arg Phe Asp Ala Phe Leu Thr Asp Ile Leu  
245 250 255  
Glu Glu His Lys Gly Lys Ile Phe Gly Glu Met Lys Asp Leu Leu Ser  
260 265 270  
Thr Leu Ile Ser Leu Lys Asn Asp Asp Ala Asp Asn Asp Gly Gly Lys  
275 280 285  
Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Val Ala  
290 295 300  
Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu  
305 310 315 320  
Ile Arg Asn Pro Lys Ile Leu Ala Gln Ala Gln Gln Glu Ile Asp Lys  
325 330 335  
Val Val Gly Arg Asp Arg Leu Val Gly Glu Leu Asp Leu Ala Gln Leu  
340 345 350  
Thr Tyr Leu Glu Ala Ile Val Lys Glu Thr Phe Arg Leu His Pro Ser  
355 360 365  
Thr Pro Leu Ser Leu Pro Arg Ile Ala Ser Glu Ser Cys Glu Ile Asn  
370 375 380  
Gly Tyr Phe Ile Pro Lys Gly Ser Thr Leu Leu Leu Asn Val Trp Ala  
385 390 395 400

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Ile Ala Arg Asp Pro Asn Ala Trp Ala Asp Pro Leu Glu Phe Arg Pro  
 405 410 415

Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Lys Val Asp Val Arg Gly  
 420 425 430

Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala  
 435 440 445

Gly Met Asn Leu Gly Ile Arg Met Val Gln Leu Met Ile Ala Thr Leu  
 450 455 460

Ile His Ala Phe Asn Trp Asp Leu Val Ser Gly Gln Leu Pro Glu Met  
 465 470 475 480

Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro  
 485 490 495

Leu Val Val His Pro Arg Pro Arg Leu Glu Ala Gln Ala Tyr Ile Gly  
 500 505 510

<210> SEQ ID NO 3  
 <211> LENGTH: 1737  
 <212> TYPE: DNA  
 <213> ORGANISM: Dianthus caryophyllus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (172)..(1674)

<400> SEQUENCE: 3

aagttcggca cgagcgtcac attcacaccg tcacattact attcaaacca ctcattttct 60

acctctcttt tctaccacc aaaacaaaac aaaacaaaa aaaacacata aaaaaactca 120

aaaaaaaatt ataatgtcac ccttagaggt aactttctac accatagtcc t atg cac 177  
 Met His  
 1

aat ctc tac tac ctc atc acc acc gtc ttc cgc ggc cac caa aaa ccg 225  
 Asn Leu Tyr Tyr Leu Ile Thr Thr Val Phe Arg Gly His Gln Lys Pro  
 5 10 15

ctt cct cca ggg cca cga cca tgg ccc atc gtg gga aac ctc cca cat 273  
 Leu Pro Pro Gly Pro Arg Pro Trp Pro Ile Val Gly Asn Leu Pro His  
 20 25 30

atg ggc cag gca ccg cac cag ggc tta gca gcc ctg gcg caa aag tat 321  
 Met Gly Gln Ala Pro His Gln Gly Leu Ala Ala Leu Ala Gln Lys Tyr  
 35 40 45 50

ggc cct cta ttg tat atg aga ctg ggg tac gtg gac gtt gtt gtg gcc 369  
 Gly Pro Leu Leu Tyr Met Arg Leu Gly Tyr Val Asp Val Val Val Ala  
 55 60 65

gcc tca gcg tct gta gcg acc cag ttt ctt aag aca cat gac cta aat 417  
 Ala Ser Ala Ser Val Ala Thr Gln Phe Leu Lys Thr His Asp Leu Asn  
 70 75 80

ttt tcg agt agg cca ccg aat tcg ggg gct aaa cac att gct tat aac 465  
 Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His Ile Ala Tyr Asn  
 85 90 95

tat caa gac ctt gtt ttt gca cct tat gga cct aaa tgg cgc atg ctt 513  
 Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Lys Trp Arg Met Leu  
 100 105 110

agg aaa att tgt tcc tta cac atg ttt tct tct aag gct ttg gac gat 561  
 Arg Lys Ile Cys Ser Leu His Met Phe Ser Ser Lys Ala Leu Asp Asp  
 115 120 125 130

ttt aga ctt gtc cgt cag gaa gaa gta tct ata ctg gta aat gcg ata 609  
 Phe Arg Leu Val Arg Gln Glu Glu Val Ser Ile Leu Val Asn Ala Ile  
 135 140 145

gca aaa gca gga aca aag cca gta caa cta gga caa cta ctc aac gtg 657  
 Ala Lys Ala Gly Thr Lys Pro Val Gln Leu Gly Gln Leu Leu Asn Val

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150	155	160	
tgc acc aca aat gcc tta tcg agg gtg atg cta ggg aag cga gtt ctc Cys Thr Thr Asn Ala Leu Ser Arg Val Met Leu Gly Lys Arg Val Leu 165	170	175	705
ggt gat ggc aca ggg aaa agc gac cca aaa gcc gag gaa ttt aag gac Gly Asp Gly Thr Gly Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Asp 180	185	190	753
atg gtg ctg gag tta atg gtt ctc acc gga gtt ttt aac att ggc gat Met Val Leu Glu Leu Met Val Leu Thr Gly Val Phe Asn Ile Gly Asp 195	200	205	801
ttt gta ccg gca ttg gaa tgt cta gac tta caa ggt gtt gca tct aaa Phe Val Pro Ala Leu Glu Cys Leu Asp Leu Gln Gly Val Ala Ser Lys 215	220	225	849
atg aag aaa tta cat aaa aga ctt gat aat ttt atg agt aac att ttg Met Lys Lys Leu His Lys Arg Leu Asp Asn Phe Met Ser Asn Ile Leu 230	235	240	897
gag gaa cac aag agt gtt gca cat caa caa aat ggt gga gat ttg cta Glu Glu His Lys Ser Val Ala His Gln Gln Asn Gly Gly Asp Leu Leu 245	250	255	945
agc att ttg ata tct ttg aag gat aat tgt gat ggt gaa ggt ggc aag Ser Ile Leu Ile Ser Leu Lys Asp Asn Cys Asp Gly Glu Gly Gly Lys 260	265	270	993
ttt agt gcc aca gaa att aag gcc ttg cta ttg gat tta ttt aca gct Phe Ser Ala Thr Glu Ile Lys Ala Leu Leu Leu Asp Leu Phe Thr Ala 275	280	285	1041
gga aca gac aca tca tct agt aca act gaa tgg gcc ata gcc gaa cta Gly Thr Asp Thr Ser Ser Thr Thr Glu Trp Ala Ile Ala Glu Leu 295	300	305	1089
att cgc cat cca aaa atc tta gcc caa gtt caa caa gaa atg gac tca Ile Arg His Pro Lys Ile Leu Ala Gln Val Gln Gln Glu Met Asp Ser 310	315	320	1137
gtc gtg ggc cga gac cga ctc ata gcc gaa gct gac ata ccg aac cta Val Val Gly Arg Asp Arg Leu Ile Ala Glu Ala Asp Ile Pro Asn Leu 325	330	335	1185
acc tac ttc caa gcc gta atc aaa gag gtt ttc cga ctt cac ccg tcc Thr Tyr Phe Gln Ala Val Ile Lys Glu Val Phe Arg Leu His Pro Ser 340	345	350	1233
acc ccg ctt tcc cta cca cgg gtc gca aac gaa tcg tgc gaa ata aac Thr Pro Leu Ser Leu Pro Arg Val Ala Asn Glu Ser Cys Glu Ile Asn 355	360	365	1281
ggg tac cac att ccc aaa aac acc act tta ttg gta aat gtg tgg gcc Gly Tyr His Ile Pro Lys Asn Thr Thr Leu Leu Val Asn Val Trp Ala 375	380	385	1329
atc gca cgc gac cct gag gtt tgg gcc gac ccg tta gag ttt aaa ccc Ile Ala Arg Asp Pro Glu Val Trp Ala Asp Pro Leu Glu Phe Lys Pro 390	395	400	1377
gaa aga ttt ttg ccg ggc ggc gaa aag ccc aat gtg gat gtg aaa gga Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Val Lys Gly 405	410	415	1425
aac gat ttt gag ctg att ccg ttc ggg gcg ggc cga cgg att tgt gct Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala 420	425	430	1473
ggg ctg agt ttg ggc ctg cgt atg gtc cag ttg atg aca gcc act ttg Gly Leu Ser Leu Gly Leu Arg Met Val Gln Leu Met Thr Ala Thr Leu 435	440	445	1521
gcc cat act tat gat tgg gcc tta gct gat ggg ctt atg ccc gaa aag Ala His Thr Tyr Asp Trp Ala Leu Ala Asp Gly Leu Met Pro Glu Lys 455	460	465	1569
ctt aac atg gat gag gct tat ggg ctt acc tta cag cgt aag gtg cca			1617

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Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Lys Val Pro  
                   470                                  475                                  480

cta atg gtc cac ccg acc cgt cgg ctc tcg gcc cgc gtt tat aat tcg          1665  
 Leu Met Val His Pro Thr Arg Arg Leu Ser Ala Arg Val Tyr Asn Ser  
                   485                                  490                                  495

ggg ttt taa agcgggtact tttggtatgt attattccgt actagtttga          1714  
 Gly Phe \*

aaaataatgt attagagaaa atg  1737

<210> SEQ ID NO 4  
 <211> LENGTH: 500  
 <212> TYPE: PRT  
 <213> ORGANISM: Dianthus caryophyllus

<400> SEQUENCE: 4

Met His Asn Leu Tyr Tyr Leu Ile Thr Thr Val Phe Arg Gly His Gln  
   1                                  5                                  10                                  15

Lys Pro Leu Pro Pro Gly Pro Arg Pro Trp Pro Ile Val Gly Asn Leu  
                   20                                  25                                  30

Pro His Met Gly Gln Ala Pro His Gln Gly Leu Ala Ala Leu Ala Gln  
                   35                                  40                                  45

Lys Tyr Gly Pro Leu Leu Tyr Met Arg Leu Gly Tyr Val Asp Val Val  
                   50                                  55                                  60

Val Ala Ala Ser Ala Ser Val Ala Thr Gln Phe Leu Lys Thr His Asp  
   65                                  70                                  75                                  80

Leu Asn Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His Ile Ala  
                   85                                  90                                  95

Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Lys Trp Arg  
                   100                                  105                                  110

Met Leu Arg Lys Ile Cys Ser Leu His Met Phe Ser Ser Lys Ala Leu  
                   115                                  120                                  125

Asp Asp Phe Arg Leu Val Arg Gln Glu Glu Val Ser Ile Leu Val Asn  
   130                                  135                                  140

Ala Ile Ala Lys Ala Gly Thr Lys Pro Val Gln Leu Gly Gln Leu Leu  
   145                                  150                                  155                                  160

Asn Val Cys Thr Thr Asn Ala Leu Ser Arg Val Met Leu Gly Lys Arg  
                   165                                  170                                  175

Val Leu Gly Asp Gly Thr Gly Lys Ser Asp Pro Lys Ala Glu Glu Phe  
                   180                                  185                                  190

Lys Asp Met Val Leu Glu Leu Met Val Leu Thr Gly Val Phe Asn Ile  
                   195                                  200                                  205

Gly Asp Phe Val Pro Ala Leu Glu Cys Leu Asp Leu Gln Gly Val Ala  
   210                                  215                                  220

Ser Lys Met Lys Lys Leu His Lys Arg Leu Asp Asn Phe Met Ser Asn  
   225                                  230                                  235                                  240

Ile Leu Glu Glu His Lys Ser Val Ala His Gln Gln Asn Gly Gly Asp  
                   245                                  250                                  255

Leu Leu Ser Ile Leu Ile Ser Leu Lys Asp Asn Cys Asp Gly Glu Gly  
                   260                                  265                                  270

Gly Lys Phe Ser Ala Thr Glu Ile Lys Ala Leu Leu Leu Asp Leu Phe  
   275                                  280                                  285

Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Thr Glu Trp Ala Ile Ala  
   290                                  295                                  300

Glu Leu Ile Arg His Pro Lys Ile Leu Ala Gln Val Gln Gln Glu Met  
   305                                  310                                  315                                  320

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Asp Ser Val Val Gly Arg Asp Arg Leu Ile Ala Glu Ala Asp Ile Pro  
 325 330 335

Asn Leu Thr Tyr Phe Gln Ala Val Ile Lys Glu Val Phe Arg Leu His  
 340 345 350

Pro Ser Thr Pro Leu Ser Leu Pro Arg Val Ala Asn Glu Ser Cys Glu  
 355 360 365

Ile Asn Gly Tyr His Ile Pro Lys Asn Thr Thr Leu Leu Val Asn Val  
 370 375 380

Trp Ala Ile Ala Arg Asp Pro Glu Val Trp Ala Asp Pro Leu Glu Phe  
 385 390 395 400

Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Val  
 405 410 415

Lys Gly Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile  
 420 425 430

Cys Ala Gly Leu Ser Leu Gly Leu Arg Met Val Gln Leu Met Thr Ala  
 435 440 445

Thr Leu Ala His Thr Tyr Asp Trp Ala Leu Ala Asp Gly Leu Met Pro  
 450 455 460

Glu Lys Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Lys  
 465 470 475 480

Val Pro Leu Met Val His Pro Thr Arg Arg Leu Ser Ala Arg Val Tyr  
 485 490 495

Asn Ser Gly Phe  
 500

<210> SEQ ID NO 5  
 <211> LENGTH: 1711  
 <212> TYPE: DNA  
 <213> ORGANISM: Antirrhinum majus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (91)..(1629)

<400> SEQUENCE: 5

cgaattcccc cccccccaca ccattcaatg cctaagtctt ccatttgccg gcctaataac 60

taaaagccca ctctttccga ccatctatac atg caa cac caa tat tat tct tta 114  
 Met Gln His Gln Tyr Tyr Ser Leu  
 1 5

att acg atg gat gat att agc ata acc agc tta ttg gtg cca tgt act 162  
 Ile Thr Met Asp Asp Ile Ser Ile Thr Ser Leu Leu Val Pro Cys Thr  
 10 15 20

ttt ata tta ggg ttc ttg ctt cta tat tcc ttc ctc aac aaa aaa gta 210  
 Phe Ile Leu Gly Phe Leu Leu Leu Tyr Ser Phe Leu Asn Lys Lys Val  
 25 30 35 40

aag cca ctg cca cct gga ccg aag cca tgg ccc atc gtc gga aat ctg 258  
 Lys Pro Leu Pro Pro Gly Pro Lys Pro Trp Pro Ile Val Gly Asn Leu  
 45 50 55

cca cat ctt ggg ccg aag ccc cac cag tcg atg gcg gcg ctg gca cgg 306  
 Pro His Leu Gly Pro Lys Pro His Gln Ser Met Ala Ala Leu Ala Arg  
 60 65 70

gtg cac ggc cca tta att cat ctg aag atg ggc ttt gtg cat gtg gtt 354  
 Val His Gly Pro Leu Ile His Leu Lys Met Gly Phe Val His Val Val  
 75 80 85

gtg gcc tcc tca gca tcc gtt gcg gag aaa ttt ctg aag gtg cat gac 402  
 Val Ala Ser Ser Ala Ser Val Ala Glu Lys Phe Leu Lys Val His Asp  
 90 95 100

gca aac ttc tcg agc agg cct ccc aat tcg ggt gca aaa cac gtg gcc 450



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Ala 105	Asn	Phe	Ser	Ser	Arg 110	Pro	Pro	Asn	Ser	Gly 115	Ala	Lys	His	Val	Ala 120	
tac	aac	tat	cag	gac	ttg	gtc	ttt	gct	cct	tat	ggc	cca	cgc	tgg	cgg	498
Tyr	Asn	Tyr	Gln	Asp 125	Leu	Val	Phe	Ala	Pro 130	Tyr	Gly	Pro	Arg	Trp 135	Arg	
atg	ctc	agg	aaa	atc	tgt	gca	ctc	cac	ctc	ttc	tcc	gcc	aaa	gcc	ttg	546
Met	Leu	Arg	Lys	Ile 140	Cys	Ala	Leu	His 145	Leu	Phe	Ser	Ala	Lys	Ala 150	Leu	
aac	gac	ttc	aca	cac	gtc	aga	cag	gat	gag	gtg	ggg	atc	ctc	act	cgc	594
Asn	Asp	Phe	Thr	His 155	Val	Arg	Gln	Asp 160	Glu	Val	Gly	Ile	Leu	Thr	Arg	
gtt	cta	gca	gat	gca	gga	gaa	acg	ccg	ttg	aaa	tta	ggg	cag	atg	atg	642
Val	Leu	Ala	Asp	Ala 170	Gly	Glu	Thr	Pro 175	Leu	Lys	Leu	Gly	Gln	Met	Met	
aac	aca	tgc	gcc	acc	aat	gca	ata	gcg	cgt	gtt	atg	ttg	ggt	cga	cgc	690
Asn	Thr	Cys	Ala	Thr 185	Asn	Ala	Ile	Ala 190	Arg	Val	Met	Leu	Gly	Arg	Arg 200	
gtg	gtt	gga	cac	gca	gac	tca	aag	gcg	gag	gag	ttt	aag	gca	atg	gta	738
Val	Val	Gly	His 205	Ala	Asp	Ser	Lys	Ala 210	Glu	Glu	Phe	Lys	Ala	Met	Val	
gtg	gag	ttg	atg	gta	tta	gct	ggt	gtg	ttc	aac	tta	ggt	gat	ttt	atc	786
Val	Glu	Leu	Met 220	Val	Leu	Ala	Gly	Val 225	Phe	Asn	Leu	Gly	Asp	Phe	Ile	
cca	cct	ctt	gaa	aaa	ttg	gat	ctt	caa	ggt	gtc	att	gct	aag	atg	aag	834
Pro	Pro	Leu	Glu	Lys 235	Leu	Asp	Leu	Gln 240	Gly	Val	Ile	Ala	Lys	Met	Lys	
aag	ctt	cac	ttg	cgt	ttc	gac	tcg	ttc	ttg	agt	aag	atc	ctt	gga	gac	882
Lys	Leu	His	Leu	Arg 250	Phe	Asp	Ser	Phe 255	Leu	Ser	Lys	Ile	Leu	Gly	Asp	
cac	aag	atc	aac	agc	tca	gat	gaa	acc	aaa	ggc	cat	tcg	gat	ttg	ttg	930
His	Lys	Ile	Asn	Ser 265	Ser	Asp	Glu	Thr 270	Lys	Gly	His	Ser	Asp	Leu	Leu 280	
aac	atg	tta	att	tct	ttg	aag	gac	gct	gat	gat	gcc	gaa	gga	ggg	agg	978
Asn	Met	Leu	Ile	Ser 285	Leu	Lys	Asp	Ala 290	Asp	Asp	Ala	Glu	Gly	Gly	Arg	
ctc	acc	gac	gta	gaa	att	aaa	gcg	ttg	ctc	ttg	aac	ttg	ttt	gct	gca	1026
Leu	Thr	Asp	Val 300	Glu	Ile	Lys	Ala	Leu 305	Leu	Leu	Asn	Leu	Phe	Ala	Ala	
gga	act	gac	aca	aca	tca	agc	act	gtg	gaa	tgg	tgc	ata	gct	gag	tta	1074
Gly	Thr	Asp	Thr 315	Thr	Ser	Ser	Thr	Val 320	Glu	Trp	Cys	Ile	Ala	Glu	Leu	
gta	cga	cat	cct	gaa	atc	ctt	gcc	caa	gtc	caa	aaa	gaa	ctc	gac	tct	1122
Val	Arg	His	Pro 330	Glu	Ile	Leu	Ala	Gln 335	Val	Gln	Lys	Glu	Leu	Asp	Ser	
gtt	gtt	ggt	aag	aat	cgg	gtg	gtg	aag	gag	gct	gat	ctg	gcc	gga	tta	1170
Val	Val	Gly	Lys 345	Asn	Arg	Val	Val	Lys 350	Glu	Ala	Asp	Leu	Ala	Gly	Leu 360	
cca	ttc	ctc	caa	gcg	gtc	gtc	aag	gaa	aat	ttc	cga	ctc	cat	ccc	tcc	1218
Pro	Phe	Leu	Gln 365	Ala	Val	Val	Lys	Glu 370	Asn	Phe	Arg	Leu	His	Pro	Ser	
acc	ccg	ctc	tcc	cta	ccg	agg	atc	gca	cat	gag	agt	tgt	gaa	gtg	aat	1266
Thr	Pro	Leu	Ser 380	Leu	Pro	Arg	Ile	Ala 385	His	Glu	Ser	Cys	Glu	Val	Asn	
gga	tac	ttg	att	cca	aag	ggt	tcg	aca	ctt	ctt	gtc	aat	gtt	tgg	gca	1314
Gly	Tyr	Leu	Ile 395	Pro	Lys	Gly	Ser	Thr 400	Leu	Leu	Val	Asn	Val	Trp	Ala	
att	gct	cgc	gat	cca	aat	gtg	tgg	gat	gaa	cca	cta	gag	ttc	cgg	cct	1362
Ile	Ala	Arg	Asp 410	Pro	Asn	Val	Trp	Asp 415	Glu	Pro	Leu	Glu	Phe	Arg	Pro	

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gaa cga ttc ttg aag ggc ggg gaa aag cct aat gtc gat gtt aga ggg	1410
Glu Arg Phe Leu Lys Gly Gly Glu Lys Pro Asn Val Asp Val Arg Gly	
425 430 435 440	
aat gat ttc gaa ttg ata ccg ttc gga gcg ggc cga aga att tgt gca	1458
Asn Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala	
445 450 455	
gga atg agc tta gga ata cgt atg gtc cag ttg ttg aca gca act ttg	1506
Gly Met Ser Leu Gly Ile Arg Met Val Gln Leu Leu Thr Ala Thr Leu	
460 465 470	
aac cat gcg ttt gac ttt gat ttg gcg gat gga cag ttg cct gaa agc	1554
Asn His Ala Phe Asp Phe Asp Leu Ala Asp Gly Gln Leu Pro Glu Ser	
475 480 485	
tta aac atg gag gaa gct tat ggg ctg acc ttg caa cga gct gac cct	1602
Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Asp Pro	
490 495 500	
ttg gta gtg cac ccg aag cct agg tag gcacctcatg tttatcaaac	1649
Leu Val Val His Pro Lys Pro Arg	
505 510	
ttaggactca tgtttagaga acctcttggt gttttatcag attgaagtgt gatgtccaag	1709
ac	1711

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 512

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Antirrhinum majus

&lt;400&gt; SEQUENCE: 6

Met Gln His Gln Tyr Tyr Ser Leu Ile Thr Met Asp Asp Ile Ser Ile	
1 5 10 15	
Thr Ser Leu Leu Val Pro Cys Thr Phe Ile Leu Gly Phe Leu Leu Leu	
20 25 30	
Tyr Ser Phe Leu Asn Lys Lys Val Lys Pro Leu Pro Pro Gly Pro Lys	
35 40 45	
Pro Trp Pro Ile Val Gly Asn Leu Pro His Leu Gly Pro Lys Pro His	
50 55 60	
Gln Ser Met Ala Ala Leu Ala Arg Val His Gly Pro Leu Ile His Leu	
65 70 75 80	
Lys Met Gly Phe Val His Val Val Val Ala Ser Ser Ala Ser Val Ala	
85 90 95	
Glu Lys Phe Leu Lys Val His Asp Ala Asn Phe Ser Ser Arg Pro Pro	
100 105 110	
Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr Gln Asp Leu Val Phe	
115 120 125	
Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile Cys Ala Leu	
130 135 140	
His Leu Phe Ser Ala Lys Ala Leu Asn Asp Phe Thr His Val Arg Gln	
145 150 155 160	
Asp Glu Val Gly Ile Leu Thr Arg Val Leu Ala Asp Ala Gly Glu Thr	
165 170 175	
Pro Leu Lys Leu Gly Gln Met Met Asn Thr Cys Ala Thr Asn Ala Ile	
180 185 190	
Ala Arg Val Met Leu Gly Arg Arg Val Val Gly His Ala Asp Ser Lys	
195 200 205	
Ala Glu Glu Phe Lys Ala Met Val Val Glu Leu Met Val Leu Ala Gly	
210 215 220	
Val Phe Asn Leu Gly Asp Phe Ile Pro Pro Leu Glu Lys Leu Asp Leu	

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225		230		235		240
Gln Gly Val Ile	Ala Lys Met Lys Lys	Leu His Leu Arg Phe Asp Ser				
	245	250	255			
Phe Leu Ser Lys	Ile Leu Gly Asp His	Lys Ile Asn Ser Ser Asp Glu				
	260	265	270			
Thr Lys Gly His	Ser Asp Leu Leu Asn Met	Leu Ile Ser Leu Lys Asp				
	275	280	285			
Ala Asp Asp Ala	Glu Gly Gly Arg Leu Thr	Asp Val Glu Ile Lys Ala				
	290	295	300			
Leu Leu Leu Asn	Leu Phe Ala Ala Gly Thr	Asp Thr Thr Ser Ser Thr				
	305	310	315			320
Val Glu Trp Cys	Ile Ala Glu Leu Val Arg	His Pro Glu Ile Leu Ala				
	325	330	335			
Gln Val Gln Lys	Glu Leu Asp Ser Val Val	Gly Lys Asn Arg Val Val				
	340	345	350			
Lys Glu Ala Asp	Leu Ala Gly Leu Pro Phe	Leu Gln Ala Val Val Lys				
	355	360	365			
Glu Asn Phe Arg	Leu His Pro Ser Thr Pro	Leu Ser Leu Pro Arg Ile				
	370	375	380			
Ala His Glu Ser	Cys Glu Val Asn Gly Tyr	Leu Ile Pro Lys Gly Ser				
	385	390	395			400
Thr Leu Leu Val	Asn Val Trp Ala Ile Ala	Arg Asp Pro Asn Val Trp				
	405	410	415			
Asp Glu Pro Leu	Glu Phe Arg Pro Glu Arg	Phe Leu Lys Gly Gly Glu				
	420	425	430			
Lys Pro Asn Val	Asp Val Arg Gly Asn Asp	Phe Glu Leu Ile Pro Phe				
	435	440	445			
Gly Ala Gly Arg	Arg Ile Cys Ala Gly Met	Ser Leu Gly Ile Arg Met				
	450	455	460			
Val Gln Leu Leu	Thr Ala Thr Leu Asn His	Ala Phe Asp Phe Asp Leu				
	465	470	475			480
Ala Asp Gly Gln	Leu Pro Glu Ser Leu Asn	Met Glu Glu Ala Tyr Gly				
	485	490	495			
Leu Thr Leu Gln	Arg Ala Asp Pro Leu Val	Val His Pro Lys Pro Arg				
	500	505	510			

<210> SEQ ID NO 7  
 <211> LENGTH: 971  
 <212> TYPE: DNA  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(813)

<400> SEQUENCE: 7

gat atg ctt agc act tta atc tcc ctt aaa gga act gat ctt gac ggt	48
Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr Asp Leu Asp Gly	
1 5 10 15	
gac gga gga agc tta acg gat act gag att aaa gcc ttg cta ttg aac	96
Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn	
20 25 30	
atg ttc aca gct gga act gac acg tca gca agt acg gtg gac tgg gct	144
Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala	
35 40 45	
ata gct gaa ctt atc cgt cac ccg gat ata atg gtt aaa gcc caa gaa	192
Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu	
50 55 60	

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gaa ctt gat att gtt gtg ggc cgt gac agg cct gtt aat gaa tca gac 240  
 Glu Leu Asp Ile Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser Asp  
 65 70 75 80  
 atc gct cag ctt cct tac ctt cag gcg gtt atc aaa gag aat ttc agg 288  
 Ile Ala Gln Leu Pro Tyr Leu Gln Ala Val Ile Lys Glu Asn Phe Arg  
 85 90 95  
 ctt cat cca cca aca cca ctc tcg tta cca cac atc gcg tca gag agc 336  
 Leu His Pro Pro Thr Pro Leu Ser Leu Pro His Ile Ala Ser Glu Ser  
 100 105 110  
 tgt gag atc aac ggc tac cat atc ccg aaa gga tcg act cta ttt gac 384  
 Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr Leu Phe Asp  
 115 120 125  
 gga cat atg ggc cta ggc cgt gac ccg gat caa tgg tcc gac ccg tta 432  
 Gly His Met Gly Leu Gly Arg Asp Pro Asp Gln Trp Ser Asp Pro Leu  
 130 135 140  
 gca ttt aaa ccc gag aga ttc tta ccc ggt ggt gaa aaa tcc ggc gtt 480  
 Ala Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Ser Gly Val  
 145 150 155 160  
 gat gtg aaa gga agc gat ttc gag cta ata ccg ttc ggg gct ggg agg 528  
 Asp Val Lys Gly Ser Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg  
 165 170 175  
 cca atc tgt gca ggt tta agt tta ggg cta cgt aca gat tta agt tgc 576  
 Pro Ile Cys Ala Gly Leu Ser Leu Gly Leu Arg Thr Asp Leu Ser Cys  
 180 185 190  
 ctt cac gcc aac gtt gct cac aag cat ttg att ggg aac ttc agc tgg 624  
 Leu His Ala Asn Val Ala His Lys His Leu Ile Gly Asn Phe Ser Trp  
 195 200 205  
 aga agt tac gcc gga caa cct gaa tat cgc agg aaa agt tta ctg ggc 672  
 Arg Ser Tyr Ala Gly Gln Pro Glu Tyr Arg Arg Lys Ser Leu Leu Gly  
 210 215 220  
 ttt aca ctg caa aga gcg gtt cct tcg gtg gta cac cct aag cca agg 720  
 Phe Thr Leu Gln Arg Ala Val Pro Ser Val Val His Pro Lys Pro Arg  
 225 230 235 240  
 ttg gcc ccg aac gtt tat gga ccc cgg gtc ggc tta aaa ttt aac ttt 768  
 Leu Ala Pro Asn Val Tyr Gly Pro Arg Val Gly Leu Lys Phe Asn Phe  
 245 250 255  
 gct tct tgg aca agg tat atg gct tgc acg aaa cta acg ttt taa 813  
 Ala Ser Trp Thr Arg Tyr Met Ala Cys Thr Lys Leu Thr Phe  
 260 265 270  
 cacaccgtag tttgatccgg agttagcttt atgtaagaac gtgtaacgcc aaatcaagcc 873  
 attatcaact accgtgagct gtttgtaccc tatctataaa tcttgaagag gaacatttca 933  
 gaactcttga ctatgtttca ggaacaaaaa aaaaaaaa 971

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 270

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;400&gt; SEQUENCE: 8

Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr Asp Leu Asp Gly  
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 Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn  
 20 25 30  
 Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala  
 35 40 45  
 Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu  
 50 55 60

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Glu	Leu	Asp	Ile	Val	Val	Gly	Arg	Asp	Arg	Pro	Val	Asn	Glu	Ser	Asp	65	70	75	80
Ile	Ala	Gln	Leu	Pro	Tyr	Leu	Gln	Ala	Val	Ile	Lys	Glu	Asn	Phe	Arg	85	90	95	
Leu	His	Pro	Pro	Thr	Pro	Leu	Ser	Leu	Pro	His	Ile	Ala	Ser	Glu	Ser	100	105	110	
Cys	Glu	Ile	Asn	Gly	Tyr	His	Ile	Pro	Lys	Gly	Ser	Thr	Leu	Phe	Asp	115	120	125	
Gly	His	Met	Gly	Leu	Gly	Arg	Asp	Pro	Asp	Gln	Trp	Ser	Asp	Pro	Leu	130	135	140	
Ala	Phe	Lys	Pro	Glu	Arg	Phe	Leu	Pro	Gly	Gly	Glu	Lys	Ser	Gly	Val	145	150	155	160
Asp	Val	Lys	Gly	Ser	Asp	Phe	Glu	Leu	Ile	Pro	Phe	Gly	Ala	Gly	Arg	165	170	175	
Pro	Ile	Cys	Ala	Gly	Leu	Ser	Leu	Gly	Leu	Arg	Thr	Asp	Leu	Ser	Cys	180	185	190	
Leu	His	Ala	Asn	Val	Ala	His	Lys	His	Leu	Ile	Gly	Asn	Phe	Ser	Trp	195	200	205	
Arg	Ser	Tyr	Ala	Gly	Gln	Pro	Glu	Tyr	Arg	Arg	Lys	Ser	Leu	Leu	Gly	210	215	220	
Phe	Thr	Leu	Gln	Arg	Ala	Val	Pro	Ser	Val	Val	His	Pro	Lys	Pro	Arg	225	230	235	240
Leu	Ala	Pro	Asn	Val	Tyr	Gly	Pro	Arg	Val	Gly	Leu	Lys	Phe	Asn	Phe	245	250	255	
Ala	Ser	Trp	Thr	Arg	Tyr	Met	Ala	Cys	Thr	Lys	Leu	Thr	Phe	260	265	270			

<210> SEQ ID NO 9  
 <211> LENGTH: 6595  
 <212> TYPE: DNA  
 <213> ORGANISM: Arabidopsis thaliana  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1478)..(1927)  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (2651)..(3091)  
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 <221> NAME/KEY: CDS  
 <222> LOCATION: (3170)..(3340)  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (3421)..(3897)

<400> SEQUENCE: 9

gtcgactctc tccctttcgc ttgctacttt ttctacataa ataatgcaa tgataaattt	60
gtgcacacat tcgtatggtt gaaacatggt aggatccaca atttatactt tatagactca	120
aaatggaaaa gaaacgtaca ttataaattt atctgcaatt tgttttctct tgctaaacta	180
gactgtataa taacctctgt atatgctatt actcgattgt aaacgtaccc cgcaagtcgc	240
aagcaaggta aataaagtat aattatattt tcacacacga aactttaatt attattttta	300
tcacttgagc attaacagta aaaaaaaaaa aaatgtgact ttaacggcga caaaaactac	360
tgatctttct ccaatattta aataatataa ttaataaacg tcttttcata cttgtatttt	420
ccgacccgag ttctgaaagt gaaaacatat ggtactagat attctcgatt tgttttgtag	480
ccactagact ctaaacagaa aaaagaagcc aaaaggacaa cgttaaaaaa gagacactgt	540
tattaaagt tagaaaccaa acggtgaaaa tccagctaca tacataaaat aaagccaagg	600

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taccaaacta atgaactgta acctcttttt tcttttcttt tttgttaaag gatttatgaa	660
ctgtaactta gaatgcttgg tttgtgggca gtgtaatata tgacacacat gcattttttt	720
tgtttgcaaa ataggaagac ttcttttttc tttatcaact tccttatttt cataaaacaa	780
aactgaaa aaagtacaga tgttctcag tacgtcacgt gtacatacat atatattaga	840
ccactatata ataagatatg aagtgttagg tttaaatcaa ttaacgaatc ccatccaaat	900
gatgaaacag ttaacaagaa atcaaaatag tttattaggg ttacaatgat tttatacttt	960
taagaaatct tagaacctat cacttacaaa tgagtaaag accattactc ctcgagaatc	1020
taaggcgctt aaggaagcat tgcaaatcgg gtgtgaaaa gatctatttt ttgaattatt	1080
tcacacaatt tcttaatgtc aattttcgat gctcccatat tctccacggt ttaaagcaag	1140
attggtggga aaggatatt ctcgcatcga ttacaatgaa atatgggttg aaaaaaaaa	1200
aaaaaaaaa ctcaatgttg caccaaaaac cagaaaactc taagttgcgc taataaaaaa	1260
aaaagttata aaccaacat caaaccaaaa cgtactaaa ctgtcccata tgagatttag	1320
ctttaataa attagtactt ctcataacga taactaaatt aaatttcct agccaagaca	1380
tacatatagt tttgattgac aaaaaaaaa aaaactcctc tatttatagc ttgtgttttg	1440
tttctcatt tttcacttac cattcaaacc caacact atg gca act cta ttt ctc	1495
Met Ala Thr Leu Phe Leu	
1 5	
aca atc ctc cta gcc act gtc ctc ttc ctc atc ctc cgt atc ttc tct	1543
Thr Ile Leu Leu Ala Thr Val Leu Phe Leu Ile Leu Arg Ile Phe Ser	
10 15 20	
cac cgt cgc aac cgc agc cac aac aac cgt ctt cca ccg ggg cca aac	1591
His Arg Arg Asn Arg Ser His Asn Asn Arg Leu Pro Pro Gly Pro Asn	
25 30 35	
cca tgg ccc atc atc gga aac ctc cct cac atg ggc act aag cct cat	1639
Pro Trp Pro Ile Ile Gly Asn Leu Pro His Met Gly Thr Lys Pro His	
40 45 50	
cga acc ctt tcc gcc atg gtt act act tac ggc cct atc ctc cac ctc	1687
Arg Thr Leu Ser Ala Met Val Thr Thr Tyr Gly Pro Ile Leu His Leu	
55 60 65 70	
cga cta ggg ttc gta gac gtc gtg gtc gcc gct tct aaa tcc gtg gcc	1735
Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser Lys Ser Val Ala	
75 80 85	
gag cag ttc ttg aaa ata cac gac gcc aat ttc gct agc cga cca cca	1783
Glu Gln Phe Leu Lys Ile His Asp Ala Asn Phe Ala Ser Arg Pro Pro	
90 95 100	
aac tca gga gcc aaa cac atg gca tat aac tat caa gat ctt gtc ttt	1831
Asn Ser Gly Ala Lys His Met Ala Tyr Asn Tyr Gln Asp Leu Val Phe	
105 110 115	
gca cct tac gga cac cga tgg aga ctg ttg aga aag att agt tct gtt	1879
Ala Pro Tyr Gly His Arg Trp Arg Leu Leu Arg Lys Ile Ser Ser Val	
120 125 130	
cat cta ttt tca gct aaa gct ctc gaa gat ttc aaa cat gtt cga cag	1927
His Leu Phe Ser Ala Lys Ala Leu Glu Asp Phe Lys His Val Arg Gln	
135 140 145 150	
gtaaaacaat tataaacggt attctcattt tctaacgcta tagctcactg gcctgtaatc	1987
atgtcatttc aatgttttga ctttttcttt atatatacat aattataatt tataattggg	2047
atttcaaacc ctatctctca ctatttcaag actagaccgg attggaattt gaacttttgt	2107
aatgaatatt agtatctgca cataaatttt atgttaaagt tgggttttct taaagtgaat	2167
ttatatatta aaaatatata aacgattggg ttttactcaa atgaatttac ataagagcta	2227
ggtataagtg caaatatgca atactgtcat tgcgtggat gtataaaagt atgatctaac	2287

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tttgatgatg ccatgaaaa attggaaagt tcagatccag aggaaacatt gcttgaatta	2347
taaaatgtat ggaccacatt gtttccttaa atggaaggtc tcacgagttt ctcaatttca	2407
gactactgat aatatatgct attatagatt ttattttctg attatttttt ttggtttaat	2467
ttaattagag taaattttta aaaagaaata tatggttttg ttaaccgtgt tttaaaattt	2527
gatagagctt ttagatcata atcataattt tttcgtatta attgtgatta tgttggacga	2587
aaatacttaa ttagtattca agaaaactct tattctaaaa acagaaataa atgaatttta	2647
cag gaa gag gtt gga acg cta acg cgg gag cta gtg cgt gtt ggc acg	2695
Glu Glu Val Gly Thr Leu Thr Arg Glu Leu Val Arg Val Gly Thr	
1 5 10 15	
aaa ccc gtg aat tta ggc cag ttg gtg aac atg tgt gta gtc aac gct	2743
Lys Pro Val Asn Leu Gly Gln Leu Val Asn Met Cys Val Val Asn Ala	
20 25 30	
cta gga cga gag atg atc gga cgg cga ctg ttc ggc gcc gac gcc gat	2791
Leu Gly Arg Glu Met Ile Gly Arg Arg Leu Phe Gly Ala Asp Ala Asp	
35 40 45	
cat aaa gct gac gag ttt cga tcg atg gtg acg gaa atg atg gct ctc	2839
His Lys Ala Asp Glu Phe Arg Ser Met Val Thr Glu Met Met Ala Leu	
50 55 60	
gcc gga gta ttt aac atc gga gat ttc gtg ccg tca ctt gat tgg tta	2887
Ala Gly Val Phe Asn Ile Gly Asp Phe Val Pro Ser Leu Asp Trp Leu	
65 70 75	
gat tta caa ggc gtc gct ggt aaa atg aaa cgg ctt cac aaa aga ttc	2935
Asp Leu Gln Gly Val Ala Gly Lys Met Lys Arg Leu His Lys Arg Phe	
80 85 90 95	
gac gct ttt cta tcg tcg att ttg aaa gag cac gaa atg aac ggt caa	2983
Asp Ala Phe Leu Ser Ser Ile Leu Lys Glu His Glu Met Asn Gly Gln	
100 105 110	
gat caa aag cat aca gat atg ctt agc act tta atc tcc ctt aaa gga	3031
Asp Gln Lys His Thr Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly	
115 120 125	
act gat ctt gac ggt gac gga gga agc tta acg gat act gag att aaa	3079
Thr Asp Leu Asp Gly Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys	
130 135 140	
gcc ttg cta ttg gtcagttttt tgacaattaa tttccttaaa aatcgtatat	3131
Ala Leu Leu Leu	
145	
aatgaaagtt agattgtttt ttttggttgt aaatacag aac atg ttc aca gct	3184
Asn Met Phe Thr Ala	
1 5	
gga act gac acg tca gca agt acg gtg gac tgg gct ata gct gaa ctt	3232
Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp Ala Ile Ala Glu Leu	
10 15 20	
atc cgt cac ccg gat ata atg gtt aaa gcc caa gaa gaa ctt gat att	3280
Ile Arg His Pro Asp Ile Met Val Lys Ala Gln Glu Glu Leu Asp Ile	
25 30 35	
gtt gtg ggc cgt gac agg cct gtt aat gaa tca gac atc gct cag ctt	3328
Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser Asp Ile Ala Gln Leu	
40 45 50	
cct tac ctt cag gtaccgtaa cccaaaccgg aatttggat tgttttggtt	3380
Pro Tyr Leu Gln	
55	
agcgagctat tgttgttaat ccggttttgg tttaaaacag gcg gtt atc aaa gag	3435
Ala Val Ile Lys Glu	
1 5	
aat ttc agg ctt cat cca cca aca cca ctc tcg tta cca cac atc gcg	3483
Asn Phe Arg Leu His Pro Pro Thr Pro Leu Ser Leu Pro His Ile Ala	

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10	15	20	
tca gag agc tgt gag atc aac ggc tac cat atc ccg aaa gga tcg act			3531
Ser Glu Ser Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr			
25	30	35	
cta ttg acg aac ata tgg gcc ata gcc cgt gac ccg gat caa tgg tcc			3579
Leu Leu Thr Asn Ile Trp Ala Ile Ala Arg Asp Pro Asp Gln Trp Ser			
40	45	50	
gac ccg tta gca ttt aaa ccc gag aga ttc tta ccc ggt ggt gaa aaa			3627
Asp Pro Leu Ala Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys			
55	60	65	
tcc ggc gtt gat gtg aaa gga agc gat ttc gag cta ata ccg ttc gga			3675
Ser Gly Val Asp Val Lys Gly Ser Asp Phe Glu Leu Ile Pro Phe Gly			
70	75	80	85
gct ggg agg aga atc tgt gcc ggt tta agt tta ggg tta cgt acg att			3723
Ala Gly Arg Arg Ile Cys Ala Gly Leu Ser Leu Gly Leu Arg Thr Ile			
90	95	100	
cag ttt ctt acg gcg acg ttg gtt caa gga ttt gat tgg gaa tta gct			3771
Gln Phe Leu Thr Ala Thr Leu Val Gln Gly Phe Asp Trp Glu Leu Ala			
105	110	115	
gga gga gtt acg ccg gag aag ctg aat atg gag gag agt tat ggg ctt			3819
Gly Gly Val Thr Pro Glu Lys Leu Asn Met Glu Glu Ser Tyr Gly Leu			
120	125	130	
aca ctg caa aga gcg gtt cct ttg gtg gta cat cct aag cca agg ttg			3867
Thr Leu Gln Arg Ala Val Pro Leu Val Val His Pro Lys Pro Arg Leu			
135	140	145	
gct ccg aac gtt tat gga ctc ggg tcg ggt taaaatttaa ctttgcttct			3917
Ala Pro Asn Val Tyr Gly Leu Gly Ser Gly			
150	155		
tggacaaggt atatggcttg cacgaaaata aagtttttaa acagcgtagt ttgatccgga			3977
gtagcttta tgtaagaacg tgtaacgcca aatcaagtca ttattaaata ttgtgagttg			4037
tttgtaacct atatataaat cttgaagagg aagatttcag aaatcttgaa tatgttttag			4097
gaaaaacatt gttttttta cagtagcgca agttgaatta aaacctattc cttacagaac			4157
caaatgcatt aataattcta gatatttttg gccaaagaca tcagattttt caatatttca			4217
tatatactag gtggaacacc accacctgca actctgcaac acatggtacg ttacacaatc			4277
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cagaaagtat gttttaattt ttaaatttga aaaaggtgta cttacaagtt cgggtgttca			4517
cacggaggag agctacaata atgaaaaagc tgactcaaga agggctatag aagaaacaag			4577
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tctaacagtg ccatttgtat ctctgaactc caacatggct cctctggttc tctaccttct			4757
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atctcttatg caactcttgt tgatgatttg caaacatctt aacctctcga aacaagattt			4997
acaaatctta ctggcttcac ttacaatttt gttcccattt ttttcttctt tggtaggtgc			5057
ctcatggtgt gtgtgcaaaa cagggtgag tgactcagtg ctacaaaaga cattagacta			5117
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tgacaatggt agggctcatt gcaactatgc agtcaatagc ttcttccaaa agaaagggtca 5237
agcttctgag tcttgtaact tcaactggtac tgccactcct accaccaccg atcccagtaa 5297
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agatattttt tctctgatgt gtgatttttt ggcacaggct atacaggatg tgcattccct 5477
tctagtgcta ggtacggctc tttgcttctc tacacattta ttttcttaat ggctttatct 5537
agaactttga aggataccat tttatttttt ttggacaaag aaggatagcc atttaatact 5597
acactttaat gttggattaa ctaacttatt atgcctatct aatggcctac actttaagtg 5657
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ttggggattg tataagtttg aacctatggt tagtgtattg acttgcgctg ctcttattgc 5837
aacggttctt tgaaaacaca ttaatgataa ataaattgaa aagtatagag atggcaattg 5897
tttcaaagc taatctttct gcttgctaact actttacata aaaaacaaaa aattaagaag 5957
attttcaaac aatacaactt ttttaccttg tcctaacaaa ttcaactcaa atgacatgtg 6017
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caaccgaaag cagtggattt gcgctctatt actccaacaa ccttctgtta accggctttt 6377
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ttctttttta ctttccggtt ttaagaaaac aattatcaat gtaaaaccaa atctactatc 6557
gatcggtttg gtacgaattc ctgcagcccg ggggatcc 6595

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&lt;210&gt; SEQ ID NO 10

&lt;211&gt; LENGTH: 150

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;400&gt; SEQUENCE: 10

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Met Ala Thr Leu Phe Leu Thr Ile Leu Leu Ala Thr Val Leu Phe Leu
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Ile Leu Arg Ile Phe Ser His Arg Arg Asn Arg Ser His Asn Asn Arg
 20             25             30
Leu Pro Pro Gly Pro Asn Pro Trp Pro Ile Ile Gly Asn Leu Pro His
 35             40             45
Met Gly Thr Lys Pro His Arg Thr Leu Ser Ala Met Val Thr Thr Tyr
 50             55             60
Gly Pro Ile Leu His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala
 65             70             75             80
Ala Ser Lys Ser Val Ala Glu Gln Phe Leu Lys Ile His Asp Ala Asn
 85             90             95
Phe Ala Ser Arg Pro Pro Asn Ser Gly Ala Lys His Met Ala Tyr Asn
100            105            110
Tyr Gln Asp Leu Val Phe Ala Pro Tyr Gly His Arg Trp Arg Leu Leu

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115	120	125
Arg Lys Ile Ser Ser Val His Leu Phe Ser Ala Lys Ala Leu Glu Asp 130 135 140		
Phe Lys His Val Arg Gln 145 150		
 <210> SEQ ID NO 11 <211> LENGTH: 147 <212> TYPE: PRT <213> ORGANISM: Arabidopsis thaliana		
 <400> SEQUENCE: 11		
Glu Glu Val Gly Thr Leu Thr Arg Glu Leu Val Arg Val Gly Thr Lys 1 5 10 15		
Pro Val Asn Leu Gly Gln Leu Val Asn Met Cys Val Val Asn Ala Leu 20 25 30		
Gly Arg Glu Met Ile Gly Arg Arg Leu Phe Gly Ala Asp Ala Asp His 35 40 45		
Lys Ala Asp Glu Phe Arg Ser Met Val Thr Glu Met Met Ala Leu Ala 50 55 60		
Gly Val Phe Asn Ile Gly Asp Phe Val Pro Ser Leu Asp Trp Leu Asp 65 70 75 80		
Leu Gln Gly Val Ala Gly Lys Met Lys Arg Leu His Lys Arg Phe Asp 85 90 95		
Ala Phe Leu Ser Ser Ile Leu Lys Glu His Glu Met Asn Gly Gln Asp 100 105 110		
Gln Lys His Thr Asp Met Leu Ser Thr Leu Ile Ser Leu Lys Gly Thr 115 120 125		
Asp Leu Asp Gly Asp Gly Gly Ser Leu Thr Asp Thr Glu Ile Lys Ala 130 135 140		
Leu Leu Leu 145		

<210> SEQ ID NO 12  
<211> LENGTH: 57  
<212> TYPE: PRT  
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 12

Asn Met Phe Thr Ala Gly Thr Asp Thr Ser Ala Ser Thr Val Asp Trp 1 5 10 15
Ala Ile Ala Glu Leu Ile Arg His Pro Asp Ile Met Val Lys Ala Gln 20 25 30
Glu Glu Leu Asp Ile Val Val Gly Arg Asp Arg Pro Val Asn Glu Ser 35 40 45
Asp Ile Ala Gln Leu Pro Tyr Leu Gln 50 55

<210> SEQ ID NO 13  
<211> LENGTH: 159  
<212> TYPE: PRT  
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 13

Ala Val Ile Lys Glu Asn Phe Arg Leu His Pro Pro Thr Pro Leu Ser 1 5 10 15
Leu Pro His Ile Ala Ser Glu Ser Cys Glu Ile Asn Gly Tyr His Ile 20 25 30

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Pro Lys Gly Ser Thr Leu Leu Thr Asn Ile Trp Ala Ile Ala Arg Asp  
 35 40 45  
 Pro Asp Gln Trp Ser Asp Pro Leu Ala Phe Lys Pro Glu Arg Phe Leu  
 50 55 60  
 Pro Gly Gly Glu Lys Ser Gly Val Asp Val Lys Gly Ser Asp Phe Glu  
 65 70 75 80  
 Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Leu Ser Leu  
 85 90 95  
 Gly Leu Arg Thr Ile Gln Phe Leu Thr Ala Thr Leu Val Gln Gly Phe  
 100 105 110  
 Asp Trp Glu Leu Ala Gly Gly Val Thr Pro Glu Lys Leu Asn Met Glu  
 115 120 125  
 Glu Ser Tyr Gly Leu Thr Leu Gln Arg Ala Val Pro Leu Val Val His  
 130 135 140  
 Pro Lys Pro Arg Leu Ala Pro Asn Val Tyr Gly Leu Gly Ser Gly  
 145 150 155

<210> SEQ ID NO 14  
 <211> LENGTH: 1748  
 <212> TYPE: DNA  
 <213> ORGANISM: Rosa hybrida  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (22)..(1563)

<400> SEQUENCE: 14

tgctcgagaaa gaagaacagc c atg ttt ctc ata gta gtg atc acc ttc ctc	51
Met Phe Leu Ile Val Val Ile Thr Phe Leu	
1 5 10	
ttc gcc gtg ttt ttg ttc cgg ctt ctt ttc tcc ggc aaa tcc caa cgc	99
Phe Ala Val Phe Leu Phe Arg Leu Leu Phe Ser Gly Lys Ser Gln Arg	
15 20 25	
cac tcg ctc cct ctc cct cct ggc ccc aaa cca tgg ccg gtg gtt ggc	147
His Ser Leu Pro Leu Pro Pro Gly Pro Lys Pro Trp Pro Val Val Gly	
30 35 40	
aac tta cct cac ttg ggc ccc ttc ccg cac cac tcc atc gcg gag ttg	195
Asn Leu Pro His Leu Gly Pro Phe Pro His His Ser Ile Ala Glu Leu	
45 50 55	
gcg aag aaa cac ggg ccg ctc atg cac ctc cgc ctc ggc tac gtt gac	243
Ala Lys Lys His Gly Pro Leu Met His Leu Arg Leu Gly Tyr Val Asp	
60 65 70	
gta gtc gtg gcg gca tca gca tcc gta gcg gcc cag ttc ttg aag act	291
Val Val Val Ala Ala Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Thr	
75 80 85 90	
cac gac gcc aat ttc tcc agc cga ccg ccc aac tcc ggc gcc aag cac	339
His Asp Ala Asn Phe Ser Ser Arg Pro Pro Asn Ser Gly Ala Lys His	
95 100 105	
ctc gcc tat aac tac cag gac ctc gtg ttc agg ccg tac ggt cca cgg	387
Leu Ala Tyr Asn Tyr Gln Asp Leu Val Phe Arg Pro Tyr Gly Pro Arg	
110 115 120	
tgg cgc atg ttc cgg aag atc agc tcc gtc cat ctg ttc tcc ggc aaa	435
Trp Arg Met Phe Arg Lys Ile Ser Ser Val His Leu Phe Ser Gly Lys	
125 130 135	
gcc ttg gat gat ctt aaa cac gtc cgg cag gag gag gta agt gtg cta	483
Ala Leu Asp Asp Leu Lys His Val Arg Gln Glu Glu Val Ser Val Leu	
140 145 150	
gcg cat gcc ttg gca aat tca ggg tca aag gta gtg aac ctg gcg caa	531
Ala His Ala Leu Ala Asn Ser Gly Ser Lys Val Val Asn Leu Ala Gln	
155 160 165 170	

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ctg ctg aac ctg tgc acg gtc aat gct cta gga agg gtg atg gta ggg Leu Leu Asn Leu Cys Thr Val Asn Ala Leu Gly Arg Val Met Val Gly 175 180 185	579
cgg agg gtt ttc ggc gac ggc agc gga ggc gac gat ccg aag gcg gac Arg Arg Val Phe Gly Asp Gly Ser Gly Gly Asp Asp Pro Lys Ala Asp 190 195 200	627
gag ttc aaa tcg atg gtg gtg gag atg atg gtg ttg gca gga gtg ttc Glu Phe Lys Ser Met Val Val Glu Met Met Val Leu Ala Gly Val Phe 205 210 215	675
aac ata ggt gac ttc atc ccc tct ctc gaa tgg ctt gac ttg caa ggc Asn Ile Gly Asp Phe Ile Pro Ser Leu Glu Trp Leu Asp Leu Gln Gly 220 225 230	723
gtg gcg tcc aag atg aag aag ctc cac aag aga ttc gac gac ttc ttg Val Ala Ser Lys Met Lys Lys Leu His Lys Arg Phe Asp Asp Phe Leu 235 240 245 250	771
aca gcc att gtc gag gac cac aag aag ggc tcc ggc acg gcg ggg cac Thr Ala Ile Val Glu Asp His Lys Lys Gly Ser Gly Thr Ala Gly His 255 260 265	819
gtc gac atg ttg acc act ctg ctc tcg ctc aag gaa gac gcc gac ggc Val Asp Met Leu Thr Thr Leu Leu Ser Leu Lys Glu Asp Ala Asp Gly 270 275 280	867
gaa gga ggc aag ctc acc gat act gaa atc aaa gct ttg ctt ttg aac Glu Gly Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn 285 290 295	915
atg ttc acg gct ggc act gat acg tca tcg agc acg gtg gaa tgg gca Met Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala 300 305 310	963
ata gct gaa ctc att cgg cac cct cat atg cta gcg cga gtt cag aaa Ile Ala Glu Leu Ile Arg His Pro His Met Leu Ala Arg Val Gln Lys 315 320 325 330	1011
gag ctt gac gat ttt gtt ggc cat gac cga ctt gtg acc gaa tcc gac Glu Leu Asp Asp Phe Val Gly His Asp Arg Leu Val Thr Glu Ser Asp 335 340 345	1059
ata ccc aac ctc cct tac ctc caa gcc gtg atc aag gaa acg ttc cga Ile Pro Asn Leu Pro Tyr Leu Gln Ala Val Ile Lys Glu Thr Phe Arg 350 355 360	1107
ctc cac cca tcc act cct ctc tcg ttg cct cgt atg gca gcc gag agt Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Ala Ala Glu Ser 365 370 375	1155
tgc gaa atc aac ggg tac cac atc ccg aaa ggc tcc aca ctc ttg gtc Cys Glu Ile Asn Gly Tyr His Ile Pro Lys Gly Ser Thr Leu Leu Val 380 385 390	1203
aat gta tgg gcc ata tcg cgt gac ccg gct gaa tgg gcc gac cca ctg Asn Val Trp Ala Ile Ser Arg Asp Pro Ala Glu Trp Ala Asp Pro Leu 395 400 405 410	1251
gag ttc aag ccc gag agg ttc ctg ccg ggg ggc gaa aag cct aat gtt Glu Phe Lys Pro Glu Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val 415 420 425	1299
gat att aga gga aac gat ttt gaa gtc ata ccc ttc ggt gcc ggg cga Asp Ile Arg Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg 430 435 440	1347
aga ata tgt gcc ggg atg agc ttg ggc ctg cgt atg gtc cat tta atg Arg Ile Cys Ala Gly Met Ser Leu Gly Leu Arg Met Val His Leu Met 445 450 455	1395
act gca aca ttg gtc cac gca ttt aat tgg gcc ttg gct gat ggg ctg Thr Ala Thr Leu Val His Ala Phe Asn Trp Ala Leu Ala Asp Gly Leu 460 465 470	1443
acc gct gag aag tta aac atg gat gaa gca tat ggg ctc act cta caa Thr Ala Glu Lys Leu Asn Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln	1491

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475	480	485	490	
cga gct gca ccg tta atg gtg cac ccg cgc acc agg ctg gcc cca cag				1539
Arg Ala Ala Pro Leu Met Val His Pro Arg Thr Arg Leu Ala Pro Gln				
	495	500	505	
gca tat aaa act tca tca tct taa ttagagagct atgttctggg tgtgcccggt				1593
Ala Tyr Lys Thr Ser Ser Ser				
	510			
ttgatgtctc catgttttct atttaggttt aaatctgtaa gataaggtga ttctatgctg				1653
aatcacaaaa gttgctatct aaattccatg tccaatgaaa acgttcttct tcccttctta				1713
taatttatga atacttatga tataggcgac agcaa				1748

<210> SEQ ID NO 15  
 <211> LENGTH: 513  
 <212> TYPE: PRT  
 <213> ORGANISM: Rosa hybrida

<400> SEQUENCE: 15

Met Phe Leu Ile Val Val Ile Thr Phe Leu Phe Ala Val Phe Leu Phe				
1	5	10	15	
Arg Leu Leu Phe Ser Gly Lys Ser Gln Arg His Ser Leu Pro Leu Pro				
	20	25	30	
Pro Gly Pro Lys Pro Trp Pro Val Val Gly Asn Leu Pro His Leu Gly				
	35	40	45	
Pro Phe Pro His His Ser Ile Ala Glu Leu Ala Lys Lys His Gly Pro				
	50	55	60	
Leu Met His Leu Arg Leu Gly Tyr Val Asp Val Val Val Ala Ala Ser				
	65	70	75	80
Ala Ser Val Ala Ala Gln Phe Leu Lys Thr His Asp Ala Asn Phe Ser				
	85	90	95	
Ser Arg Pro Pro Asn Ser Gly Ala Lys His Leu Ala Tyr Asn Tyr Gln				
	100	105	110	
Asp Leu Val Phe Arg Pro Tyr Gly Pro Arg Trp Arg Met Phe Arg Lys				
	115	120	125	
Ile Ser Ser Val His Leu Phe Ser Gly Lys Ala Leu Asp Asp Leu Lys				
	130	135	140	
His Val Arg Gln Glu Glu Val Ser Val Leu Ala His Ala Leu Ala Asn				
	145	150	155	160
Ser Gly Ser Lys Val Val Asn Leu Ala Gln Leu Leu Asn Leu Cys Thr				
	165	170	175	
Val Asn Ala Leu Gly Arg Val Met Val Gly Arg Arg Val Phe Gly Asp				
	180	185	190	
Gly Ser Gly Gly Asp Asp Pro Lys Ala Asp Glu Phe Lys Ser Met Val				
	195	200	205	
Val Glu Met Met Val Leu Ala Gly Val Phe Asn Ile Gly Asp Phe Ile				
	210	215	220	
Pro Ser Leu Glu Trp Leu Asp Leu Gln Gly Val Ala Ser Lys Met Lys				
	225	230	235	240
Lys Leu His Lys Arg Phe Asp Asp Phe Leu Thr Ala Ile Val Glu Asp				
	245	250	255	
His Lys Lys Gly Ser Gly Thr Ala Gly His Val Asp Met Leu Thr Thr				
	260	265	270	
Leu Leu Ser Leu Lys Glu Asp Ala Asp Gly Glu Gly Gly Lys Leu Thr				
	275	280	285	
Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Met Phe Thr Ala Gly Thr				

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290	295	300
Asp Thr Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg 305 310 315 320		
His Pro His Met Leu Ala Arg Val Gln Lys Glu Leu Asp Asp Phe Val 325 330 335		
Gly His Asp Arg Leu Val Thr Glu Ser Asp Ile Pro Asn Leu Pro Tyr 340 345 350		
Leu Gln Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro 355 360 365		
Leu Ser Leu Pro Arg Met Ala Ala Glu Ser Cys Glu Ile Asn Gly Tyr 370 375 380		
His Ile Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ser 385 390 395 400		
Arg Asp Pro Ala Glu Trp Ala Asp Pro Leu Glu Phe Lys Pro Glu Arg 405 410 415		
Phe Leu Pro Gly Gly Glu Lys Pro Asn Val Asp Ile Arg Gly Asn Asp 420 425 430		
Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met 435 440 445		
Ser Leu Gly Leu Arg Met Val His Leu Met Thr Ala Thr Leu Val His 450 455 460		
Ala Phe Asn Trp Ala Leu Ala Asp Gly Leu Thr Ala Glu Lys Leu Asn 465 470 475 480		
Met Asp Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ala Pro Leu Met 485 490 495		
Val His Pro Arg Thr Arg Leu Ala Pro Gln Ala Tyr Lys Thr Ser Ser 500 505 510		

Ser

<210> SEQ ID NO 16  
 <211> LENGTH: 1660  
 <212> TYPE: DNA  
 <213> ORGANISM: Chrysanthemum  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (4)..(1530)

&lt;400&gt; SEQUENCE: 16

aaa atg acc att tta gct ttc gta ttt tac gcc ctc atc ctc ggg tca Met Thr Ile Leu Ala Phe Val Phe Tyr Ala Leu Ile Leu Gly Ser 1 5 10 15	48
gta ctc tat gta ttt ctt aac tta agt tca cgt aaa tcc gcc aga ctc Val Leu Tyr Val Phe Leu Asn Leu Ser Ser Arg Lys Ser Ala Arg Leu 20 25 30	96
cca ccc ggg cca aca cca tgg cct ata gtc ggg aac tta cca cac ctt Pro Pro Gly Pro Thr Pro Trp Pro Ile Val Gly Asn Leu Pro His Leu 35 40 45	144
ggc cca atc cca cac cac gca ctc gcg gcc tta gcc aag aag tac ggg Gly Pro Ile Pro His His Ala Leu Ala Ala Leu Ala Lys Lys Tyr Gly 50 55 60	192
cca ttg atg cac ctg cgg ctc ggg tgt gtg gac gtg gtt gtg gcc gcg Pro Leu Met His Leu Arg Leu Gly Cys Val Asp Val Val Val Ala Ala 65 70 75	240
tct gct tcc gta gct gca cag ttt tta aaa gtt cac gac gca aat ttt Ser Ala Ser Val Ala Ala Gln Phe Leu Lys Val His Asp Ala Asn Phe 80 85 90 95	288
gct agt agg ccg cca aat tct ggc gcg aaa cat gtg gcg tat aat tat	336

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Ala Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr	
100 105 110	
cag gat ctt gtg ttt gca cct tat ggt cca agg tgg cgt ttg tta agg	384
Gln Asp Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Leu Leu Arg	
115 120 125	
aag att tgt tcg gtc cat ttg ttt tct gct aaa gca ctt gat gat ttt	432
Lys Ile Cys Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe	
130 135 140	
cgt cat gtt cga cag gag gag gta gca gtc cta acc cgc gta cta ctg	480
Arg His Val Arg Gln Glu Glu Val Ala Val Leu Thr Arg Val Leu Leu	
145 150 155	
agt gct gga aac tca ccg gta cag ctt ggc caa cta ctt aac gtg tgt	528
Ser Ala Gly Asn Ser Pro Val Gln Leu Gly Gln Leu Leu Asn Val Cys	
160 165 170 175	
gcc aca aac gcc tta gca cgg gta atg tta ggt agg aga gtt ttc gga	576
Ala Thr Asn Ala Leu Ala Arg Val Met Leu Gly Arg Arg Val Phe Gly	
180 185 190	
gac gga att gac agg tca gcc aat gag ttc aaa gat atg gta gta gag	624
Asp Gly Ile Asp Arg Ser Ala Asn Glu Phe Lys Asp Met Val Val Glu	
195 200 205	
tta atg gta tta gca gga gaa ttt aac ctt ggt gac ttt att cct gta	672
Leu Met Val Leu Ala Gly Glu Phe Asn Leu Gly Asp Phe Ile Pro Val	
210 215 220	
ctt gac cta ttc gac cta caa ggc att act aaa aaa atg aag aag ctt	720
Leu Asp Leu Phe Asp Leu Gln Gly Ile Thr Lys Lys Met Lys Lys Leu	
225 230 235	
cat gtt cgg ttc gat tca ttt ctt agt aag atc gtt gag gag cat aaa	768
His Val Arg Phe Asp Ser Phe Leu Ser Lys Ile Val Glu Glu His Lys	
240 245 250 255	
acg gca cct ggt ggg ttg ggt cat act gat ttg ctg agc acg ttg att	816
Thr Ala Pro Gly Gly Leu Gly His Thr Asp Leu Leu Ser Thr Leu Ile	
260 265 270	
tca ctt aaa gat gat gct gat att gaa ggt ggg aag ctt aca gat act	864
Ser Leu Lys Asp Asp Ala Asp Ile Glu Gly Gly Lys Leu Thr Asp Thr	
275 280 285	
gaa atc aaa gct ttg ctt ctg aat tta ttc gct gcg gga aca gac aca	912
Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ala Ala Gly Thr Asp Thr	
290 295 300	
tcc tct agt aca gta gaa tgg gca ata gcc gaa ctc att cgt cat cca	960
Ser Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg His Pro	
305 310 315	
caa ata tta aaa caa gcc cga gaa gag ata gac gct gta gtt ggt caa	1008
Gln Ile Leu Lys Gln Ala Arg Glu Glu Ile Asp Ala Val Val Gly Gln	
320 325 330 335	
gac cgg ctt gta aca gaa ttg gac ttg agc caa cta aca tac ctc cag	1056
Asp Arg Leu Val Thr Glu Leu Asp Leu Ser Gln Leu Thr Tyr Leu Gln	
340 345 350	
gct ctt gtg aaa gag gtg ttt agg ctc cac cct tca acg cca ctc tcc	1104
Ala Leu Val Lys Glu Val Phe Arg Leu His Pro Ser Thr Pro Leu Ser	
355 360 365	
tta cca aga ata tca tcc gag agt tgt gag gtc gat ggg tat tat atc	1152
Leu Pro Arg Ile Ser Ser Glu Ser Cys Glu Val Asp Gly Tyr Tyr Ile	
370 375 380	
cct aag gga tcc aca ctc ctc gtt aac gtg tgg gcc att gcg cga gac	1200
Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp	
385 390 395	
cca aaa atg tgg gcg gat cct ctt gaa ttt agg cct tct cgg ttt tta	1248
Pro Lys Met Trp Ala Asp Pro Leu Glu Phe Arg Pro Ser Arg Phe Leu	
400 405 410 415	





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Asp Leu Phe Asp Leu Gln Gly Ile Thr Lys Lys Met Lys Lys Leu His  
 225 230 235 240

Val Arg Phe Asp Ser Phe Leu Ser Lys Ile Val Glu Glu His Lys Thr  
 245 250 255

Ala Pro Gly Gly Leu Gly His Thr Asp Leu Leu Ser Thr Leu Ile Ser  
 260 265 270

Leu Lys Asp Asp Ala Asp Ile Glu Gly Gly Lys Leu Thr Asp Thr Glu  
 275 280 285

Ile Lys Ala Leu Leu Leu Asn Leu Phe Ala Ala Gly Thr Asp Thr Ser  
 290 295 300

Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg His Pro Gln  
 305 310 315 320

Ile Leu Lys Gln Ala Arg Glu Glu Ile Asp Ala Val Val Gly Gln Asp  
 325 330 335

Arg Leu Val Thr Glu Leu Asp Leu Ser Gln Leu Thr Tyr Leu Gln Ala  
 340 345 350

Leu Val Lys Glu Val Phe Arg Leu His Pro Ser Thr Pro Leu Ser Leu  
 355 360 365

Pro Arg Ile Ser Ser Glu Ser Cys Glu Val Asp Gly Tyr Tyr Ile Pro  
 370 375 380

Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp Pro  
 385 390 395 400

Lys Met Trp Ala Asp Pro Leu Glu Phe Arg Pro Ser Arg Phe Leu Pro  
 405 410 415

Gly Gly Glu Lys Pro Gly Ala Asp Val Arg Gly Asn Asp Phe Glu Val  
 420 425 430

Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu Gly  
 435 440 445

Leu Arg Met Val Gln Leu Leu Ile Ala Thr Leu Val Gln Thr Phe Asp  
 450 455 460

Trp Glu Leu Ala Asn Gly Leu Glu Pro Glu Met Leu Asn Met Glu Glu  
 465 470 475 480

Ala Tyr Gly Leu Thr Leu Gln Arg Ala Ala Pro Leu Met Val His Pro  
 485 490 495

Lys Pro Arg Leu Ala Pro His Val Tyr Glu Ser Ile  
 500 505

<210> SEQ ID NO 18  
 <211> LENGTH: 1815  
 <212> TYPE: DNA  
 <213> ORGANISM: Torenia  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (107)..(1633)

<400> SEQUENCE: 18

ctaaattaat taataaat acacacgacg agatgtatgt aatgtaatgt aatattatta 60

catacatcat caccgaatac gcacgctact accactgcga ttagcc atg agt ccc 115  
 Met Ser Pro  
 1

tta gcc ttg atg atc ata agt acc tta tta ggg ttt ctc cta tac cac 163  
 Leu Ala Leu Met Ile Ile Ser Thr Leu Leu Gly Phe Leu Leu Tyr His  
 5 10 15

tct ctt cgc tta cta ctc ttc tcc ggc caa ggt cgc cga cta cta cca 211  
 Ser Leu Arg Leu Leu Leu Phe Ser Gly Gln Gly Arg Arg Leu Leu Pro  
 20 25 30 35

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cca ggt cca cgc ccg tgg ccg ctg gtg gga aat ctc ccg cac tta ggc	259
Pro Gly Pro Arg Pro Trp Pro Leu Val Gly Asn Leu Pro His Leu Gly	
40 45 50	
ccg aag cca cac gcc tcc atg gcc gag ctc gcg cga gcc tac gga ccc	307
Pro Lys Pro His Ala Ser Met Ala Glu Leu Ala Arg Ala Tyr Gly Pro	
55 60 65	
ctc atg cac cta aag atg ggg ttc gtc cac gtc gtg gtg gct tcg tcg	355
Leu Met His Leu Lys Met Gly Phe Val His Val Val Val Ala Ser Ser	
70 75 80	
gcg agc gcg gcg gag cag tgc ctg agg gtt cac gac gcg aat ttc ttg	403
Ala Ser Ala Ala Glu Gln Cys Leu Arg Val His Asp Ala Asn Phe Leu	
85 90 95	
agc agg cca ccc aac tcc ggc gcc aag cac gtc gct tac aac tac gag	451
Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr Asn Tyr Glu	
100 105 110 115	
gac ttg gtt ttc aga ccg tac ggt ccc aag tgg agg ctg ttg agg aag	499
Asp Leu Val Phe Arg Pro Tyr Gly Pro Lys Trp Arg Leu Leu Arg Lys	
120 125 130	
ata tgc gct cag cat att ttc tcc gtc aag gct atg gat gac ttc agg	547
Ile Cys Ala Gln His Ile Phe Ser Val Lys Ala Met Asp Asp Phe Arg	
135 140 145	
cgc gtc aga gag gaa gag gtg gcc atc ctg agt cgc gct cta gca ggc	595
Arg Val Arg Glu Glu Glu Val Ala Ile Leu Ser Arg Ala Leu Ala Gly	
150 155 160	
aaa agg gcc gta ccc ata ggc caa atg ctc aac gtg tgc gcc aca aac	643
Lys Arg Ala Val Pro Ile Gly Gln Met Leu Asn Val Cys Ala Thr Asn	
165 170 175	
gcc cta tct cgc gtc atg atg ggg cgg cgc gtg gtg ggc cac gcg gat	691
Ala Leu Ser Arg Val Met Met Gly Arg Arg Val Val Gly His Ala Asp	
180 185 190 195	
gga acc aac gac gcc aag gcg gag gag ttc aaa gcc atg gtc gtc gag	739
Gly Thr Asn Asp Ala Lys Ala Glu Glu Phe Lys Ala Met Val Val Glu	
200 205 210	
ctc atg gtc ctc tcc ggc gtc ttc aac atc ggt gat ttc atc ccc ttc	787
Leu Met Val Leu Ser Gly Val Phe Asn Ile Gly Asp Phe Ile Pro Phe	
215 220 225	
ctc gag cct ctc gac ttg cag gga gtg gct tcc aag atg aag aaa ctc	835
Leu Glu Pro Leu Asp Leu Gln Gly Val Ala Ser Lys Met Lys Lys Leu	
230 235 240	
cac gcg cgg ttc gat gca ttc ttg acc gag att gta cga gag cgt tgt	883
His Ala Arg Phe Asp Ala Phe Leu Thr Glu Ile Val Arg Glu Arg Cys	
245 250 255	
cat ggg cag atc aac aac agt ggt gct cat cag gat gat ttg ctt agc	931
His Gly Gln Ile Asn Asn Ser Gly Ala His Gln Asp Asp Leu Leu Ser	
260 265 270 275	
acg ttg att tcg ttc aaa ggg ctt gac gat ggc gat ggt tcc agg ctc	979
Thr Leu Ile Ser Phe Lys Gly Leu Asp Asp Gly Asp Gly Ser Arg Leu	
280 285 290	
act gac aca gaa atc aag gcg ctg ctc ttg aac ctt ttg gac acg acg	1027
Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Leu Asp Thr Thr	
295 300 305	
tcg agc acg gtg gaa tgg gcc gta gcc gaa ctc cta cgc cac cct aag	1075
Ser Ser Thr Val Glu Trp Ala Val Ala Glu Leu Leu Arg His Pro Lys	
310 315 320	
aca tta gcc caa gtc cgg caa gag ctc gac tcg gtc gtg ggt aag aac	1123
Thr Leu Ala Gln Val Arg Gln Glu Leu Asp Ser Val Val Gly Lys Asn	
325 330 335	
agg ctc gtg tcc gag acc gat ctg aat cag ctg ccc tat cta caa gct	1171
Arg Leu Val Ser Glu Thr Asp Leu Asn Gln Leu Pro Tyr Leu Gln Ala	

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340	345	350	355	
gtc gtc aaa gaa act ttc cgc ctc cat cct ccg acg ccg ctc tct cta				1219
Val Val Lys Glu Thr Phe Arg Leu His Pro Pro Thr Pro Leu Ser Leu				
	360	365	370	
ccg aga ctc gcg gaa gat gat tgc gag atc gac gga tac ctc atc ccc				1267
Pro Arg Leu Ala Glu Asp Asp Cys Glu Ile Asp Gly Tyr Leu Ile Pro				
	375	380	385	
aag ggc tcg acc ctt ctg gtg aac gtt tgg gcc ata gcc cgc gat ccc				1315
Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp Pro				
	390	395	400	
aag gtt tgg gcc gat ccg ttg gag ttt agg ccc gaa cga ttc ttg acg				1363
Lys Val Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu Thr				
	405	410	415	
ggc gga gaa aag gcc gac gtc gat gtc aag ggg aac gat ttc gaa gtg				1411
Gly Gly Glu Lys Ala Asp Val Asp Val Lys Gly Asn Asp Phe Glu Val				
	420	425	430	435
ata ccg ttc ggg gcg ggt cgt agg atc tgc gct ggc gtt ggc ttg gga				1459
Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Val Gly Leu Gly				
	440	445	450	
ata cgt atg gtc caa ctg ttg acg gcg agt ttg atc cat gca ttc gat				1507
Ile Arg Met Val Gln Leu Leu Thr Ala Ser Leu Ile His Ala Phe Asp				
	455	460	465	
ctg gac ctt gct aat ggg ctt ttg gcc caa aat ctg aac atg gaa gaa				1555
Leu Asp Leu Ala Asn Gly Leu Leu Ala Gln Asn Leu Asn Met Glu Glu				
	470	475	480	
gca tat ggg ctt acg cta caa cgg gct gag cct ttg ttg gtc cac cct				1603
Ala Tyr Gly Leu Thr Leu Gln Arg Ala Glu Pro Leu Leu Val His Pro				
	485	490	495	
agg ccg cgg ttg gcc act cat gtc tat taa ttaaattagg cctaaactac				1653
Arg Pro Arg Leu Ala Thr His Val Tyr				
	500	505		
gatgaatgac ccatttaacg ttaataagag ttttcaatth atgtgagttt gcatggtatg 1713				
gtatggtatg gtgcttgtaa taaattgtat ctgttaggtg tgttcattga tgataaatct 1773				
agtttgtact gctgctcaaa aaaaaaaaaa aaaaaaaaaa aa 1815				

<210> SEQ ID NO 19  
 <211> LENGTH: 508  
 <212> TYPE: PRT  
 <213> ORGANISM: Torenia

<400> SEQUENCE: 19

Met Ser Pro Leu Ala Leu Met Ile Ile Ser Thr Leu Leu Gly Phe Leu				
1	5	10	15	
Leu Tyr His Ser Leu Arg Leu Leu Leu Phe Ser Gly Gln Gly Arg Arg				
	20	25	30	
Leu Leu Pro Pro Gly Pro Arg Pro Trp Pro Leu Val Gly Asn Leu Pro				
	35	40	45	
His Leu Gly Pro Lys Pro His Ala Ser Met Ala Glu Leu Ala Arg Ala				
	50	55	60	
Tyr Gly Pro Leu Met His Leu Lys Met Gly Phe Val His Val Val Val				
	65	70	75	80
Ala Ser Ser Ala Ser Ala Ala Glu Gln Cys Leu Arg Val His Asp Ala				
	85	90	95	
Asn Phe Leu Ser Arg Pro Pro Asn Ser Gly Ala Lys His Val Ala Tyr				
	100	105	110	
Asn Tyr Glu Asp Leu Val Phe Arg Pro Tyr Gly Pro Lys Trp Arg Leu				
	115	120	125	

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Leu Arg Lys Ile Cys Ala Gln His Ile Phe Ser Val Lys Ala Met Asp  
 130 135 140  
 Asp Phe Arg Arg Val Arg Glu Glu Glu Val Ala Ile Leu Ser Arg Ala  
 145 150 155 160  
 Leu Ala Gly Lys Arg Ala Val Pro Ile Gly Gln Met Leu Asn Val Cys  
 165 170 175  
 Ala Thr Asn Ala Leu Ser Arg Val Met Met Gly Arg Arg Val Val Gly  
 180 185 190  
 His Ala Asp Gly Thr Asn Asp Ala Lys Ala Glu Glu Phe Lys Ala Met  
 195 200 205  
 Val Val Glu Leu Met Val Leu Ser Gly Val Phe Asn Ile Gly Asp Phe  
 210 215 220  
 Ile Pro Phe Leu Glu Pro Leu Asp Leu Gln Gly Val Ala Ser Lys Met  
 225 230 235 240  
 Lys Lys Leu His Ala Arg Phe Asp Ala Phe Leu Thr Glu Ile Val Arg  
 245 250 255  
 Glu Arg Cys His Gly Gln Ile Asn Asn Ser Gly Ala His Gln Asp Asp  
 260 265 270  
 Leu Leu Ser Thr Leu Ile Ser Phe Lys Gly Leu Asp Asp Gly Asp Gly  
 275 280 285  
 Ser Arg Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn Leu Leu  
 290 295 300  
 Asp Thr Thr Ser Ser Thr Val Glu Trp Ala Val Ala Glu Leu Leu Arg  
 305 310 315 320  
 His Pro Lys Thr Leu Ala Gln Val Arg Gln Glu Leu Asp Ser Val Val  
 325 330 335  
 Gly Lys Asn Arg Leu Val Ser Glu Thr Asp Leu Asn Gln Leu Pro Tyr  
 340 345 350  
 Leu Gln Ala Val Val Lys Glu Thr Phe Arg Leu His Pro Pro Thr Pro  
 355 360 365  
 Leu Ser Leu Pro Arg Leu Ala Glu Asp Asp Cys Glu Ile Asp Gly Tyr  
 370 375 380  
 Leu Ile Pro Lys Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala  
 385 390 395 400  
 Arg Asp Pro Lys Val Trp Ala Asp Pro Leu Glu Phe Arg Pro Glu Arg  
 405 410 415  
 Phe Leu Thr Gly Gly Glu Lys Ala Asp Val Asp Val Lys Gly Asn Asp  
 420 425 430  
 Phe Glu Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Val  
 435 440 445  
 Gly Leu Gly Ile Arg Met Val Gln Leu Leu Thr Ala Ser Leu Ile His  
 450 455 460  
 Ala Phe Asp Leu Asp Leu Ala Asn Gly Leu Leu Ala Gln Asn Leu Asn  
 465 470 475 480  
 Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln Arg Ala Glu Pro Leu Leu  
 485 490 495  
 Val His Pro Arg Pro Arg Leu Ala Thr His Val Tyr  
 500 505

&lt;210&gt; SEQ ID NO 20

&lt;211&gt; LENGTH: 1824

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Jap. Morning Glory

&lt;220&gt; FEATURE:

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&lt;221&gt; NAME/KEY: CDS

&lt;222&gt; LOCATION: (2)..(1555)

&lt;400&gt; SEQUENCE: 20

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g agc tta acc tta att ttc tgc act tta gtt ttt gca atc ttt cta tat      49
  Ser Leu Thr Leu Ile Phe Cys Thr Leu Val Phe Ala Ile Phe Leu Tyr
    1           5           10          15

ttt ctt att ctc agg gtg aaa cag cgt tac cct tta cct ctc cca ccc      97
Phe Leu Ile Leu Arg Val Lys Gln Arg Tyr Pro Leu Pro Leu Pro Pro
          20           25           30

gga cca aaa cca tgg ccg gtg tta gga aac ctt ccc cac ctg ggc aag      145
Gly Pro Lys Pro Trp Pro Val Leu Gly Asn Leu Pro His Leu Gly Lys
          35           40           45

aag cct cac cag tcg att gcg gcc atg gct gag agg tac ggc ccc ctc      193
Lys Pro His Gln Ser Ile Ala Ala Met Ala Glu Arg Tyr Gly Pro Leu
          50           55           60

atg cac ctc cgc cta gga ttc gtg gac gtg gtt gtg gcc gcc tcc gcc      241
Met His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser Ala
          65           70           75           80

gcc gtg gcc gct cag ttc ttg aaa gtt cac gac tcg aac ttc tcc aac      289
Ala Val Ala Ala Gln Phe Leu Lys Val His Asp Ser Asn Phe Ser Asn
          85           90           95

cgg ccg ccg aac tcc ggc gcg gaa cac att gct tat aac tat caa gac      337
Arg Pro Pro Asn Ser Gly Ala Glu His Ile Ala Tyr Asn Tyr Gln Asp
          100          105          110

ctc gtc ttc gcg ccc tac ggc ccg ccg tgg cgc atg ctt agg aag atc      385
Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile
          115          120          125

acc tcc gtg cat ctc ttc tcg gcc aag gcg ttg gat gac ttc tgc cat      433
Thr Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe Cys His
          130          135          140

gtt cgc cag gaa gag gtt gca act ctg aca cgc agt cta gca agt gca      481
Val Arg Gln Glu Glu Val Ala Thr Leu Thr Arg Ser Leu Ala Ser Ala
          145          150          155          160

ggc aaa act cca gta aaa cta ggg cag tta cta aac gtg tgc acc acg      529
Gly Lys Thr Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr Thr
          165          170          175

aac gcc cta gct cgt gta atg cta ggg ccg aag gtc ttt aat gac gga      577
Asn Ala Leu Ala Arg Val Met Leu Gly Arg Lys Val Phe Asn Asp Gly
          180          185          190

ggt agc aag agc gac cca aag gcg gag gag ttc aag tcg atg gtg gag      625
Gly Ser Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Ser Met Val Glu
          195          200          205

gag atg atg gtg ttg gcc gga agt ttt aac atc ggc gat ttc att ccg      673
Glu Met Met Val Leu Ala Gly Ser Phe Asn Ile Gly Asp Phe Ile Pro
          210          215          220

gtc ttg ggt tgg ttt gac gtt cag ggt atc gta ggg aag atg aag aaa      721
Val Leu Gly Trp Phe Asp Val Gln Gly Ile Val Gly Lys Met Lys Lys
          225          230          235          240

cta cac gcg cgt ttt gat gcg ttc ttg aac acc att cta gag gaa cac      769
Leu His Ala Arg Phe Asp Ala Phe Leu Asn Thr Ile Leu Glu Glu His
          245          250          255

aaa tgt gtc aac aat caa cac acg acg ttg tcg aaa gat gtg gac ttc      817
Lys Cys Val Asn Asn Gln His Thr Thr Leu Ser Lys Asp Val Asp Phe
          260          265          270

ttg agc acc cta att agg ctc aaa gat aat ggg gct gat atg gat tgt      865
Leu Ser Thr Leu Ile Arg Leu Lys Asp Asn Gly Ala Asp Met Asp Cys
          275          280          285

gaa gag gga aaa ctc acc gac act gaa att aag gct ttg ctc ttg aac      913
Glu Glu Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn

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290	295	300	
ctg ttc aca gct ggg act gat aca tca tct agc act gtg gag tgg gca Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala 305 310 315 320			961
atc gca gaa cta cta cgc aac cca aaa atc tta aac caa gca caa caa Ile Ala Glu Leu Leu Arg Asn Pro Lys Ile Leu Asn Gln Ala Gln Gln 325 330 335			1009
gag ctt gac tta gtg gtg ggt caa aat cag cta gtc aca gaa tct gac Glu Leu Asp Leu Val Val Gly Gln Asn Gln Leu Val Thr Glu Ser Asp 340 345 350			1057
tta acc gat cta cct ttc ctg caa gca ata gtg aag gag acc ttc agg Leu Thr Asp Leu Pro Phe Leu Gln Ala Ile Val Lys Glu Thr Phe Arg 355 360 365			1105
cta cac cca tcc acc cca ctc tct ctt cca aga atg gga gct cag ggt Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Gly Ala Gln Gly 370 375 380			1153
tgc gag atc aat ggc tac ttc atc ccc aaa ggc gca acg ctt ttg gtc Cys Glu Ile Asn Gly Tyr Phe Ile Pro Lys Gly Ala Thr Leu Leu Val 385 390 395 400			1201
aac gtt tgg gcc ata gct cgt gat ccc aat gtg tgg aca aat cct ctt Asn Val Trp Ala Ile Ala Arg Asp Pro Asn Val Trp Thr Asn Pro Leu 405 410 415			1249
gag ttc aac cca cac cga ttc ttg cct ggt gga gaa aag ccc aac gtg Glu Phe Asn Pro His Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val 420 425 430			1297
gat att aaa ggg aat gac ttt gaa gtg att cct ttt gga gcc ggg cgt Asp Ile Lys Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg 435 440 445			1345
aga ata tgc tct ggg atg agt ttg ggg ata agg atg gtt cac ctg ttg Arg Ile Cys Ser Gly Met Ser Leu Gly Ile Arg Met Val His Leu Leu 450 455 460			1393
gtt gca act ttg gtg cat gct ttt gat tgg gat ttg gtg aat gga caa Val Ala Thr Leu Val His Ala Phe Asp Trp Asp Leu Val Asn Gly Gln 465 470 475 480			1441
tct gta gag acg ctc aat atg gag gaa gct tat ggt ctc acc ctt caa Ser Val Glu Thr Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln 485 490 495			1489
cga gct gtt cct ttg atg ttg cat cca aag ccc aga tta caa cca cat Arg Ala Val Pro Leu Met Leu His Pro Lys Pro Arg Leu Gln Pro His 500 505 510			1537
ctc tat act ctc aat taa attgcaattt gattttggtg attatacaat Leu Tyr Thr Leu Asn 515			1585
tataatcgag ggacatagga tccccattta tttatattca gttataagag acttccaaca			1645
aaggtctagc tttcgacctt aaaagttgta aaagaggtcc tacatatgta aaagcccgcc			1705
aaaggaaaac tggttgtatt caattccgct aggccttgtc cgaaagacct catgaagact			1765
acaagggtca tatataatgg taaacccagt gtatttggtg taaaaaaaa aaaaaaaaa			1824
<210> SEQ ID NO 21			
<211> LENGTH: 517			
<212> TYPE: PRT			
<213> ORGANISM: Jap. Morning Glory			
<400> SEQUENCE: 21			
Ser Leu Thr Leu Ile Phe Cys Thr Leu Val Phe Ala Ile Phe Leu Tyr 1 5 10 15			
Phe Leu Ile Leu Arg Val Lys Gln Arg Tyr Pro Leu Pro Leu Pro Pro 20 25 30			

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Gly Pro Lys Pro Trp Pro Val Leu Gly Asn Leu Pro His Leu Gly Lys  
                   35                                  40                                  45

Lys Pro His Gln Ser Ile Ala Ala Met Ala Glu Arg Tyr Gly Pro Leu  
           50                                  55                                  60

Met His Leu Arg Leu Gly Phe Val Asp Val Val Val Ala Ala Ser Ala  
       65                                  70                                  75                                  80

Ala Val Ala Ala Gln Phe Leu Lys Val His Asp Ser Asn Phe Ser Asn  
                                   85                                  90                                  95

Arg Pro Pro Asn Ser Gly Ala Glu His Ile Ala Tyr Asn Tyr Gln Asp  
                   100                                  105                                  110

Leu Val Phe Ala Pro Tyr Gly Pro Arg Trp Arg Met Leu Arg Lys Ile  
           115                                  120                                  125

Thr Ser Val His Leu Phe Ser Ala Lys Ala Leu Asp Asp Phe Cys His  
       130                                  135                                  140

Val Arg Gln Glu Glu Val Ala Thr Leu Thr Arg Ser Leu Ala Ser Ala  
   145                                  150                                  155                                  160

Gly Lys Thr Pro Val Lys Leu Gly Gln Leu Leu Asn Val Cys Thr Thr  
                   165                                  170                                  175

Asn Ala Leu Ala Arg Val Met Leu Gly Arg Lys Val Phe Asn Asp Gly  
                   180                                  185                                  190

Gly Ser Lys Ser Asp Pro Lys Ala Glu Glu Phe Lys Ser Met Val Glu  
           195                                  200                                  205

Glu Met Met Val Leu Ala Gly Ser Phe Asn Ile Gly Asp Phe Ile Pro  
   210                                  215                                  220

Val Leu Gly Trp Phe Asp Val Gln Gly Ile Val Gly Lys Met Lys Lys  
   225                                  230                                  235                                  240

Leu His Ala Arg Phe Asp Ala Phe Leu Asn Thr Ile Leu Glu Glu His  
                   245                                  250                                  255

Lys Cys Val Asn Asn Gln His Thr Thr Leu Ser Lys Asp Val Asp Phe  
                   260                                  265                                  270

Leu Ser Thr Leu Ile Arg Leu Lys Asp Asn Gly Ala Asp Met Asp Cys  
           275                                  280                                  285

Glu Glu Gly Lys Leu Thr Asp Thr Glu Ile Lys Ala Leu Leu Leu Asn  
   290                                  295                                  300

Leu Phe Thr Ala Gly Thr Asp Thr Ser Ser Ser Thr Val Glu Trp Ala  
   305                                  310                                  315                                  320

Ile Ala Glu Leu Leu Arg Asn Pro Lys Ile Leu Asn Gln Ala Gln Gln  
                   325                                  330                                  335

Glu Leu Asp Leu Val Val Gly Gln Asn Gln Leu Val Thr Glu Ser Asp  
           340                                  345                                  350

Leu Thr Asp Leu Pro Phe Leu Gln Ala Ile Val Lys Glu Thr Phe Arg  
           355                                  360                                  365

Leu His Pro Ser Thr Pro Leu Ser Leu Pro Arg Met Gly Ala Gln Gly  
           370                                  375                                  380

Cys Glu Ile Asn Gly Tyr Phe Ile Pro Lys Gly Ala Thr Leu Leu Val  
   385                                  390                                  395                                  400

Asn Val Trp Ala Ile Ala Arg Asp Pro Asn Val Trp Thr Asn Pro Leu  
                   405                                  410                                  415

Glu Phe Asn Pro His Arg Phe Leu Pro Gly Gly Glu Lys Pro Asn Val  
                   420                                  425                                  430

Asp Ile Lys Gly Asn Asp Phe Glu Val Ile Pro Phe Gly Ala Gly Arg  
           435                                  440                                  445

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Arg Ile Cys Ser Gly Met Ser Leu Gly Ile Arg Met Val His Leu Leu  
 450 455 460

Val Ala Thr Leu Val His Ala Phe Asp Trp Asp Leu Val Asn Gly Gln  
 465 470 475 480

Ser Val Glu Thr Leu Asn Met Glu Glu Ala Tyr Gly Leu Thr Leu Gln  
 485 490 495

Arg Ala Val Pro Leu Met Leu His Pro Lys Pro Arg Leu Gln Pro His  
 500 505 510

Leu Tyr Thr Leu Asn  
 515

<210> SEQ ID NO 22  
 <211> LENGTH: 1667  
 <212> TYPE: DNA  
 <213> ORGANISM: Gentian  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(1431)

<400> SEQUENCE: 22

ccc atc ctc gga aac atc ccc cat ctc ggc tcc aaa ccg cac caa aca 48  
 Pro Ile Leu Gly Asn Ile Pro His Leu Gly Ser Lys Pro His Gln Thr  
 1 5 10 15

ctc gcg gaa atg gcg aaa acc tac ggt ccg ctc atg cac ttg aag ttc 96  
 Leu Ala Glu Met Ala Lys Thr Tyr Gly Pro Leu Met His Leu Lys Phe  
 20 25 30

ggg ctt aag gac gcg gtg gtg gcg tcg tct gcg tcg gtg gca gag cag 144  
 Gly Leu Lys Asp Ala Val Val Ala Ser Ser Ala Ser Val Ala Glu Gln  
 35 40 45

ttt ctg aag aaa cac gac gtg aat ttc tcg aac cgg ccg cca aac tcc 192  
 Phe Leu Lys Lys His Asp Val Asn Phe Ser Asn Arg Pro Pro Asn Ser  
 50 55 60

ggg gcc aaa cat ata gct tat aac tat cag gac ctg gta ttc gct ccc 240  
 Gly Ala Lys His Ile Ala Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro  
 65 70 75 80

tat gga ccc cgg tgg cgg ttg ctt agg aaa atc tgt tcc gtc cat ctt 288  
 Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys Ile Cys Ser Val His Leu  
 85 90 95

ttc tcg tct aag gcc ttg gat gac ttt cag cat gtt cga cat gag gag 336  
 Phe Ser Ser Lys Ala Leu Asp Asp Phe Gln His Val Arg His Glu Glu  
 100 105 110

ata tgc atc ctt ata cga gca ata gcg agt ggc ggt cat gct ccg gtg 384  
 Ile Cys Ile Leu Ile Arg Ala Ile Ala Ser Gly Gly His Ala Pro Val  
 115 120 125

aat tta ggc aag tta tta gga gtg tgc aca acc aat gcc ctg gca aga 432  
 Asn Leu Gly Lys Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg  
 130 135 140

gtg atg ctt gga aga aga gta ttc gaa ggc gac ggc ggc gag aat ccg 480  
 Val Met Leu Gly Arg Arg Val Phe Glu Gly Asp Gly Gly Glu Asn Pro  
 145 150 155 160

cat gcc gac gag ttt aaa tca atg gtg gtg gag att atg gtg tta gcc 528  
 His Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala  
 165 170 175

ggt gca ttc aac ttg ggt gat ttc atc ccg gtt cta gat tgg ttc gat 576  
 Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Val Leu Asp Trp Phe Asp  
 180 185 190

ttg caa gga att gct ggt aaa atg aag aaa ctt cat gcc cgt ttc gac 624  
 Leu Gln Gly Ile Ala Gly Lys Met Lys Lys Leu His Ala Arg Phe Asp  
 195 200 205

aag ttt tta aat ggg atc cta gaa gat cgt aaa tct aac ggc tct aat 672



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Lys	Phe	Leu	Asn	Gly	Ile	Leu	Glu	Asp	Arg	Lys	Ser	Asn	Gly	Ser	Asn	
210						215					220					
gga	gct	gaa	caa	tac	gtg	gac	ttg	ctc	agt	gtg	ttg	atc	tct	ctt	caa	720
Gly	Ala	Glu	Gln	Tyr	Val	Asp	Leu	Leu	Ser	Val	Leu	Ile	Ser	Leu	Gln	
225					230					235					240	
gat	agt	aat	atc	gac	ggg	ggg	gac	gaa	gga	acc	aaa	ctc	aca	gat	act	768
Asp	Ser	Asn	Ile	Asp	Gly	Gly	Asp	Glu	Gly	Thr	Lys	Leu	Thr	Asp	Thr	
				245						250					255	
gaa	atc	aaa	gct	ctc	ctt	ttg	aac	ttg	ttc	ata	gcc	gga	aca	gac	act	816
Glu	Ile	Lys	Ala	Leu	Leu	Leu	Asn	Leu	Phe	Ile	Ala	Gly	Thr	Asp	Thr	
			260							265					270	
tca	tca	agt	act	gta	gaa	tgg	gcc	atg	gca	gaa	cta	atc	cga	aac	cca	864
Ser	Ser	Ser	Thr	Val	Glu	Trp	Ala	Met	Ala	Glu	Leu	Ile	Arg	Asn	Pro	
			275						280						285	
aag	tta	cta	gtc	caa	gcc	caa	gaa	gag	cta	gac	aga	gta	gtc	ggg	ccg	912
Lys	Leu	Leu	Val	Gln	Ala	Gln	Glu	Glu	Leu	Asp	Arg	Val	Val	Gly	Pro	
			290							295					300	
aac	cga	ttc	gta	acc	gaa	tct	gat	ctt	cct	caa	ctg	aca	ttc	ctt	caa	960
Asn	Arg	Phe	Val	Thr	Glu	Ser	Asp	Leu	Pro	Gln	Leu	Thr	Phe	Leu	Gln	
					310					315					320	
gcc	gtc	atc	aaa	gag	act	ttc	agg	ctt	cat	cca	tcc	acc	cca	ctc	tct	1008
Ala	Val	Ile	Lys	Glu	Thr	Phe	Arg	Leu	His	Pro	Ser	Thr	Pro	Leu	Ser	
				325						330					335	
ctt	cca	cga	atg	gcg	gcg	gag	gac	tgt	gag	atc	aat	ggg	tat	tat	gtc	1056
Leu	Pro	Arg	Met	Ala	Ala	Glu	Asp	Cys	Glu	Ile	Asn	Gly	Tyr	Tyr	Val	
				340						345					350	
tca	gaa	ggg	tcg	aca	ttg	ctc	gtc	aat	gtg	tgg	gcc	ata	gct	cgf	gat	1104
Ser	Glu	Gly	Ser	Thr	Leu	Leu	Val	Asn	Val	Trp	Ala	Ile	Ala	Arg	Asp	
				355						360					365	
cca	aat	gcg	tgg	gcc	aat	cca	cta	gat	ttc	aac	ccg	act	cgf	ttc	ttg	1152
Pro	Asn	Ala	Trp	Ala	Asn	Pro	Leu	Asp	Phe	Asn	Pro	Thr	Arg	Phe	Leu	
				370											380	
gcc	ggg	gga	gag	aag	cct	aat	ggt	gat	ggt	aaa	ggg	aat	gat	ttt	gaa	1200
Ala	Gly	Gly	Glu	Lys	Pro	Asn	Val	Asp	Val	Lys	Gly	Asn	Asp	Phe	Glu	
					390										400	
gtg	ata	cct	ttc	ggg	gct	ggg	cgf	agg	ata	tgt	gcc	gga	atg	agc	tta	1248
Val	Ile	Pro	Phe	Gly	Ala	Gly	Arg	Arg	Ile	Cys	Ala	Gly	Met	Ser	Leu	
					405										415	
ggg	ata	cgf	atg	ggt	caa	cta	gta	acg	gct	tcg	tta	ggt	cat	tcg	ttt	1296
Gly	Ile	Arg	Met	Val	Gln	Leu	Val	Thr	Ala	Ser	Leu	Val	His	Ser	Phe	
				420											430	
gat	tgg	gct	ttg	ttg	gat	gga	ctt	aaa	ccc	gag	aag	ctt	gac	atg	gag	1344
Asp	Trp	Ala	Leu	Leu	Asp	Gly	Leu	Lys	Pro	Glu	Lys	Leu	Asp	Met	Glu	
															445	
gaa	ggg	tat	gga	cta	acg	ctt	caa	cga	gct	tca	cct	tta	atc	gtc	cat	1392
Glu	Gly	Tyr	Gly	Leu	Thr	Leu	Gln	Arg	Ala	Ser	Pro	Leu	Ile	Val	His	
															460	
cca	aag	ccg	agg	ctc	tcg	gct	caa	ggt	tat	tgt	atg	taa	caagtttgtg			1441
Pro	Lys	Pro	Arg	Leu	Ser	Ala	Gln	Val	Tyr	Cys	Met					
															475	
aagccagtct	gatttcagtt	ggatttgtag	ttattttatg	atcatttggt	atattttttt											1501
gtatttcggt	tgaatacaat	aaaggaag	tggatcgtct	gctgtataat	agcgacgttt											1561
taacgtggtg	tgatagtacc	gtggtttact	aaaacgatgt	cgtttgattt	tttatagtat											1621
taaaaaata	aacagctgga	ttttgaacca	aaaaaaaaa	aaaaaa												1667

<210> SEQ ID NO 23

<211> LENGTH: 476

<212> TYPE: PRT

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&lt;213&gt; ORGANISM: Gentian

&lt;400&gt; SEQUENCE: 23

Pro Ile Leu Gly Asn Ile Pro His Leu Gly Ser Lys Pro His Gln Thr  
 1 5 10 15  
 Leu Ala Glu Met Ala Lys Thr Tyr Gly Pro Leu Met His Leu Lys Phe  
 20 25 30  
 Gly Leu Lys Asp Ala Val Val Ala Ser Ser Ala Ser Val Ala Glu Gln  
 35 40 45  
 Phe Leu Lys Lys His Asp Val Asn Phe Ser Asn Arg Pro Pro Asn Ser  
 50 55 60  
 Gly Ala Lys His Ile Ala Tyr Asn Tyr Gln Asp Leu Val Phe Ala Pro  
 65 70 75 80  
 Tyr Gly Pro Arg Trp Arg Leu Leu Arg Lys Ile Cys Ser Val His Leu  
 85 90 95  
 Phe Ser Ser Lys Ala Leu Asp Asp Phe Gln His Val Arg His Glu Glu  
 100 105 110  
 Ile Cys Ile Leu Ile Arg Ala Ile Ala Ser Gly Gly His Ala Pro Val  
 115 120 125  
 Asn Leu Gly Lys Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg  
 130 135 140  
 Val Met Leu Gly Arg Arg Val Phe Glu Gly Asp Gly Gly Glu Asn Pro  
 145 150 155 160  
 His Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala  
 165 170 175  
 Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Val Leu Asp Trp Phe Asp  
 180 185 190  
 Leu Gln Gly Ile Ala Gly Lys Met Lys Lys Leu His Ala Arg Phe Asp  
 195 200 205  
 Lys Phe Leu Asn Gly Ile Leu Glu Asp Arg Lys Ser Asn Gly Ser Asn  
 210 215 220  
 Gly Ala Glu Gln Tyr Val Asp Leu Leu Ser Val Leu Ile Ser Leu Gln  
 225 230 235 240  
 Asp Ser Asn Ile Asp Gly Gly Asp Glu Gly Thr Lys Leu Thr Asp Thr  
 245 250 255  
 Glu Ile Lys Ala Leu Leu Leu Asn Leu Phe Ile Ala Gly Thr Asp Thr  
 260 265 270  
 Ser Ser Ser Thr Val Glu Trp Ala Met Ala Glu Leu Ile Arg Asn Pro  
 275 280 285  
 Lys Leu Leu Val Gln Ala Gln Glu Glu Leu Asp Arg Val Val Gly Pro  
 290 295 300  
 Asn Arg Phe Val Thr Glu Ser Asp Leu Pro Gln Leu Thr Phe Leu Gln  
 305 310 315 320  
 Ala Val Ile Lys Glu Thr Phe Arg Leu His Pro Ser Thr Pro Leu Ser  
 325 330 335  
 Leu Pro Arg Met Ala Ala Glu Asp Cys Glu Ile Asn Gly Tyr Tyr Val  
 340 345 350  
 Ser Glu Gly Ser Thr Leu Leu Val Asn Val Trp Ala Ile Ala Arg Asp  
 355 360 365  
 Pro Asn Ala Trp Ala Asn Pro Leu Asp Phe Asn Pro Thr Arg Phe Leu  
 370 375 380  
 Ala Gly Gly Glu Lys Pro Asn Val Asp Val Lys Gly Asn Asp Phe Glu  
 385 390 395 400

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Val Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys Ala Gly Met Ser Leu  
 405 410 415  
 Gly Ile Arg Met Val Gln Leu Val Thr Ala Ser Leu Val His Ser Phe  
 420 425 430  
 Asp Trp Ala Leu Leu Asp Gly Leu Lys Pro Glu Lys Leu Asp Met Glu  
 435 440 445  
 Glu Gly Tyr Gly Leu Thr Leu Gln Arg Ala Ser Pro Leu Ile Val His  
 450 455 460  
 Pro Lys Pro Arg Leu Ser Ala Gln Val Tyr Cys Met  
 465 470 475

<210> SEQ ID NO 24  
 <211> LENGTH: 1214  
 <212> TYPE: DNA  
 <213> ORGANISM: Lisianthus  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (2)..(1093)

<400> SEQUENCE: 24

t cgc atc ctc acg cga tct ata gcg agt gct ggg gaa aat ccg att aac 49  
 Arg Ile Leu Thr Arg Ser Ile Ala Ser Ala Gly Glu Asn Pro Ile Asn  
 1 5 10 15  
 tta ggt caa tta ctc ggg gtg tgt acc aca aat gct ctg gcg aga gtg 97  
 Leu Gly Gln Leu Leu Gly Val Cys Thr Thr Asn Ala Leu Ala Arg Val  
 20 25 30  
 atg ctt gga agg agg gta ttc ggc gat ggg agc ggc ggc gta gat cct 145  
 Met Leu Gly Arg Arg Val Phe Gly Asp Gly Ser Gly Gly Val Asp Pro  
 35 40 45  
 cag gcg gac gag ttc aaa tcc atg gtg gtg gaa atc atg gtg ttg gcc 193  
 Gln Ala Asp Glu Phe Lys Ser Met Val Val Glu Ile Met Val Leu Ala  
 50 55 60  
 ggc gcg ttt aat cta ggt gat ttt att ccc gct ctt gat tgg ttc gat 241  
 Gly Ala Phe Asn Leu Gly Asp Phe Ile Pro Ala Leu Asp Trp Phe Asp  
 65 70 75 80  
 ctg cag gga att acg gca aaa atg aag aaa gtt cac gct cgt ttc gat 289  
 Leu Gln Gly Ile Thr Ala Lys Met Lys Lys Val His Ala Arg Phe Asp  
 85 90 95  
 gcg ttc tta gac gcg atc ctt gag gag cac aaa tcc aac ggc tct cgc 337  
 Ala Phe Leu Asp Ala Ile Leu Glu His Lys Ser Asn Gly Ser Arg  
 100 105 110  
 gga gct aag caa cac gtt gac ttg ctg agt atg ttg atc tcc ctt caa 385  
 Gly Ala Lys Gln His Val Asp Leu Leu Ser Met Leu Ile Ser Leu Gln  
 115 120 125  
 gat aat aac att gat ggt gaa agt ggc gcc aaa ctc act gat aca gaa 433  
 Asp Asn Asn Ile Asp Gly Glu Ser Gly Ala Lys Leu Thr Asp Thr Glu  
 130 135 140  
 atc aaa gct ttg ctt ctg aac ttg ttc acg gct gga aca gac acg tca 481  
 Ile Lys Ala Leu Leu Leu Asn Leu Phe Thr Ala Gly Thr Asp Thr Ser  
 145 150 155 160  
 tca agt act gtg gag tgg gca atc gca gag cta atc cga aac cca gaa 529  
 Ser Ser Thr Val Glu Trp Ala Ile Ala Glu Leu Ile Arg Asn Pro Glu  
 165 170 175  
 gta ttg gtt caa gcc caa caa gag ctc gat aga gta gtt ggg cca agt 577  
 Val Leu Val Gln Ala Gln Gln Glu Leu Asp Arg Val Val Gly Pro Ser  
 180 185 190  
 cgt ctt gtg acc gaa tct gat ctg cct caa ttg gca ttc ctt caa gct 625  
 Arg Leu Val Thr Glu Ser Asp Leu Pro Gln Leu Ala Phe Leu Gln Ala  
 195 200 205  
 gtc atc aaa gag act ttc aga ctt cat cca tcc act cca ctc tct ctt 673

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Val	Ile	Lys	Glu	Thr	Phe	Arg	Leu	His	Pro	Ser	Thr	Pro	Leu	Ser	Leu		
210						215					220						
cca	cga	atg	gct	tca	gag	ggt	tgt	gaa	atc	aat	gga	tac	tcc	atc	cca	721	
Pro	Arg	Met	Ala	Ser	Glu	Gly	Cys	Glu	Ile	Asn	Gly	Tyr	Ser	Ile	Pro		
225					230					235					240		
aag	ggt	tcg	aca	ttg	ctc	ggt	aac	gta	tgg	tcc	ata	gcc	cgt	gat	cct	769	
Lys	Gly	Ser	Thr	Leu	Leu	Val	Asn	Val	Trp	Ser	Ile	Ala	Arg	Asp	Pro		
				245					250					255			
agt	ata	tgg	gcc	gac	cca	tta	gaa	ttt	agg	ccg	gca	cgt	ttc	ttg	ccc	817	
Ser	Ile	Trp	Ala	Asp	Pro	Leu	Glu	Phe	Arg	Pro	Ala	Arg	Phe	Leu	Pro		
			260					265					270				
ggc	gga	gaa	aag	ccc	aat	ggt	gat	gtg	aga	ggc	aat	gat	ttt	gag	gtc	865	
Gly	Gly	Glu	Lys	Pro	Asn	Val	Asp	Val	Arg	Gly	Asn	Asp	Phe	Glu	Val		
		275					280					285					
ata	cca	ttt	ggt	gct	gga	cgt	agg	ata	tgt	gct	gga	atg	agc	ttg	ggt	913	
Ile	Pro	Phe	Gly	Ala	Gly	Arg	Arg	Ile	Cys	Ala	Gly	Met	Ser	Leu	Gly		
	290					295					300						
tta	aga	atg	ggt	caa	ctt	tcg	aca	gct	act	ttg	ggt	cat	tcg	ttt	aat	961	
Leu	Arg	Met	Val	Gln	Leu	Ser	Thr	Ala	Thr	Leu	Val	His	Ser	Phe	Asn		
305					310					315					320		
tgg	gat	ttg	ctg	aat	ggg	atg	agc	cca	gat	aaa	ctt	gac	atg	gaa	gaa	1009	
Trp	Asp	Leu	Leu	Asn	Gly	Met	Ser	Pro	Asp	Lys	Leu	Asp	Met	Glu	Glu		
				325					330					335			
gct	tat	ggg	ctt	aca	ttg	caa	cgg	gct	tca	cct	ttg	att	gtc	cac	cca	1057	
Ala	Tyr	Gly	Leu	Thr	Leu	Gln	Arg	Ala	Ser	Pro	Leu	Ile	Val	His	Pro		
			340					345					350				
aag	ccc	agg	ctt	gct	agc	tct	atg	tat	ggt	aaa	tga	aattatgctg				1103	
Lys	Pro	Arg	Leu	Ala	Ser	Ser	Met	Tyr	Val	Lys							
		355					360										
tgccaataat	tccttattta	tagcaggaaa	tgatcatcttg	aattatgtgt	aatgttcttc											1163	
taactttcga	tggaagtgca	aaacaagttt	tattaaaaaa	aaaaaaaaa	a											1214	

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 363

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Lisianthus

&lt;400&gt; SEQUENCE: 25

Arg	Ile	Leu	Thr	Arg	Ser	Ile	Ala	Ser	Ala	Gly	Glu	Asn	Pro	Ile	Asn		
1				5					10					15			
Leu	Gly	Gln	Leu	Leu	Gly	Val	Cys	Thr	Thr	Asn	Ala	Leu	Ala	Arg	Val		
			20					25					30				
Met	Leu	Gly	Arg	Arg	Val	Phe	Gly	Asp	Gly	Ser	Gly	Gly	Val	Asp	Pro		
		35					40					45					
Gln	Ala	Asp	Glu	Phe	Lys	Ser	Met	Val	Val	Glu	Ile	Met	Val	Leu	Ala		
		50				55					60						
Gly	Ala	Phe	Asn	Leu	Gly	Asp	Phe	Ile	Pro	Ala	Leu	Asp	Trp	Phe	Asp		
		65			70					75					80		
Leu	Gln	Gly	Ile	Thr	Ala	Lys	Met	Lys	Lys	Val	His	Ala	Arg	Phe	Asp		
			85						90					95			
Ala	Phe	Leu	Asp	Ala	Ile	Leu	Glu	Glu	His	Lys	Ser	Asn	Gly	Ser	Arg		
		100						105					110				
Gly	Ala	Lys	Gln	His	Val	Asp	Leu	Leu	Ser	Met	Leu	Ile	Ser	Leu	Gln		
		115					120					125					
Asp	Asn	Asn	Ile	Asp	Gly	Glu	Ser	Gly	Ala	Lys	Leu	Thr	Asp	Thr	Glu		
		130				135					140						
Ile	Lys	Ala	Leu	Leu	Leu	Asn	Leu	Phe	Thr	Ala	Gly	Thr	Asp	Thr	Ser		

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145		150		155		160									
Ser	Ser	Thr	Val	Glu	Trp	Ala	Ile	Ala	Glu	Leu	Ile	Arg	Asn	Pro	Glu
				165					170					175	
Val	Leu	Val	Gln	Ala	Gln	Gln	Glu	Leu	Asp	Arg	Val	Val	Gly	Pro	Ser
			180					185					190		
Arg	Leu	Val	Thr	Glu	Ser	Asp	Leu	Pro	Gln	Leu	Ala	Phe	Leu	Gln	Ala
		195					200					205			
Val	Ile	Lys	Glu	Thr	Phe	Arg	Leu	His	Pro	Ser	Thr	Pro	Leu	Ser	Leu
	210					215					220				
Pro	Arg	Met	Ala	Ser	Glu	Gly	Cys	Glu	Ile	Asn	Gly	Tyr	Ser	Ile	Pro
225					230					235				240	
Lys	Gly	Ser	Thr	Leu	Leu	Val	Asn	Val	Trp	Ser	Ile	Ala	Arg	Asp	Pro
				245					250					255	
Ser	Ile	Trp	Ala	Asp	Pro	Leu	Glu	Phe	Arg	Pro	Ala	Arg	Phe	Leu	Pro
			260					265					270		
Gly	Gly	Glu	Lys	Pro	Asn	Val	Asp	Val	Arg	Gly	Asn	Asp	Phe	Glu	Val
		275					280					285			
Ile	Pro	Phe	Gly	Ala	Gly	Arg	Arg	Ile	Cys	Ala	Gly	Met	Ser	Leu	Gly
	290					295					300				
Leu	Arg	Met	Val	Gln	Leu	Ser	Thr	Ala	Thr	Leu	Val	His	Ser	Phe	Asn
305				310						315					320
Trp	Asp	Leu	Leu	Asn	Gly	Met	Ser	Pro	Asp	Lys	Leu	Asp	Met	Glu	Glu
				325					330					335	
Ala	Tyr	Gly	Leu	Thr	Leu	Gln	Arg	Ala	Ser	Pro	Leu	Ile	Val	His	Pro
			340					345					350		
Lys	Pro	Arg	Leu	Ala	Ser	Ser	Met	Tyr	Val	Lys					
	355						360								

<210> SEQ ID NO 26  
 <211> LENGTH: 1757  
 <212> TYPE: DNA  
 <213> ORGANISM: Petunia sp.  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (35)..(1525)  
 <400> SEQUENCE: 26

ccg	ttg	ctgt	cgag	aaa	aca	gaa	aga	agag	aaaa	atg	gac	tac	gtg	aat	att	ttg	55
										Met	Asp	Tyr	Val	Asn	Ile	Leu	
										1						5	
ctg	gga	ctg	ttt	ttc	act	tgg	ttc	ttg	gtg	aat	gga	ctc	atg	tca	ctt	103	
Leu	Gly	Leu	Phe	Phe	Thr	Trp	Phe	Leu	Val	Asn	Gly	Leu	Met	Ser	Leu		
		10					15					20					
cga	aga	aga	aaa	atc	tct	aag	aaa	ctt	cca	cca	ggt	cca	ttt	cct	ttg	151	
Arg	Arg	Arg	Lys	Ile	Ser	Lys	Lys	Leu	Pro	Pro	Gly	Pro	Phe	Pro	Leu		
		25				30					35						
cct	atc	atc	gga	aat	ctt	cac	tta	ctt	ggt	aat	cat	cct	cac	aaa	tca	199	
Pro	Ile	Ile	Gly	Asn	Leu	His	Leu	Leu	Gly	Asn	His	Pro	His	Lys	Ser		
	40				45				50					55			
ctt	gct	caa	ctt	gca	aaa	att	cat	ggt	cct	att	atg	aat	ctc	aaa	tta	247	
Leu	Ala	Gln	Leu	Ala	Lys	Ile	His	Gly	Pro	Ile	Met	Asn	Leu	Lys	Leu		
				60				65					70				
ggc	caa	cta	aac	aca	gtg	gtc	att	tca	tca	tca	gtc	gtg	gca	aga	gaa	295	
Gly	Gln	Leu	Asn	Thr	Val	Val	Ile	Ser	Ser	Ser	Val	Val	Ala	Arg	Glu		
			75				80						85				
gtc	ttg	caa	aaa	caa	gac	tta	aca	ttt	tcc	aat	agg	ttt	gtc	ccg	gac	343	
Val	Leu	Gln	Lys	Gln	Asp	Leu	Thr	Phe	Ser	Asn	Arg	Phe	Val	Pro	Asp		
		90					95					100					

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gta gtc cat gtc cga aat cac tcc gat ttt tct gtt gtt tgg tta cca	391
Val Val His Val Arg Asn His Ser Asp Phe Ser Val Val Trp Leu Pro	
105 110 115	
gtc aat tct cga tgg aaa acg ctt cgc aaa atc atg aac tct agc atc	439
Val Asn Ser Arg Trp Lys Thr Leu Arg Lys Ile Met Asn Ser Ser Ile	
120 125 130 135	
ttt tct ggt aac aag ctt gat ggt aat caa cat ctg agg tct aaa aag	487
Phe Ser Gly Asn Lys Leu Asp Gly Asn Gln His Leu Arg Ser Lys Lys	
140 145 150	
gtc caa gag tta att gat tat tgt caa aag tgt gcc aag aat ggc gaa	535
Val Gln Glu Leu Ile Asp Tyr Cys Gln Lys Cys Ala Lys Asn Gly Glu	
155 160 165	
gca gtg gat ata gga aga gca act ttt gga act act ttg aat ttg cta	583
Ala Val Asp Ile Gly Arg Ala Thr Phe Gly Thr Thr Leu Asn Leu Leu	
170 175 180	
tcc aac acc att ttc tct aaa gat ttg act aat ccg ttt tct gat tct	631
Ser Asn Thr Ile Phe Ser Lys Asp Leu Thr Asn Pro Phe Ser Asp Ser	
185 190 195	
gct aaa gag ttt aag gaa ttg gtt tgg aac att atg gtt gag gct gga	679
Ala Lys Glu Phe Lys Glu Leu Val Trp Asn Ile Met Val Glu Ala Gly	
200 205 210 215	
aaa ccc aat ttg gtg gac tac ttt cct ttc ctt gag aaa att gat ccg	727
Lys Pro Asn Leu Val Asp Tyr Phe Pro Phe Leu Glu Lys Ile Asp Pro	
220 225 230	
caa ggt ata aag cga cgc atg act aat aat ttt act aag ttt ctt ggc	775
Gln Gly Ile Lys Arg Arg Met Thr Asn Asn Phe Thr Lys Phe Leu Gly	
235 240 245	
ctt atc agc ggt ttg att gat gac cgg tta aag gaa agg aat cta agg	823
Leu Ile Ser Gly Leu Ile Asp Asp Arg Leu Lys Glu Arg Asn Leu Arg	
250 255 260	
gac aat gca aat att gat gtt tta gac gcc ctt ctc aac att agc caa	871
Asp Asn Ala Asn Ile Asp Val Leu Asp Ala Leu Asn Ile Ser Gln	
265 270 275	
gag aac cca gaa gag att gac agg aat caa atc gag cag ttg tgt ctg	919
Glu Asn Pro Glu Glu Ile Asp Arg Asn Gln Ile Glu Gln Leu Cys Leu	
280 285 290 295	
gac ttg ttt gca gca ggg act gat act aca tcg aat acc ttg gag tgg	967
Asp Leu Phe Ala Ala Gly Thr Asp Thr Thr Ser Asn Thr Leu Glu Trp	
300 305 310	
gca atg gca gaa cta ctt cag aat cca cac aca ttg cag aaa gca caa	1015
Ala Met Ala Glu Leu Leu Gln Asn Pro His Thr Leu Gln Lys Ala Gln	
315 320 325	
gaa gaa ctt gca caa gtc att ggt aaa ggc aaa caa gta gaa gaa gca	1063
Glu Glu Leu Ala Gln Val Ile Gly Lys Gly Lys Gln Val Glu Glu Ala	
330 335 340	
gat gtt gga cga cta cct tac ttg cga tgc ata gtg aaa gaa acc tta	1111
Asp Val Gly Arg Leu Pro Tyr Leu Arg Cys Ile Val Lys Glu Thr Leu	
345 350 355	
cga ata cac cca gcg gct cct ctc tta att cca cgt aaa gtg gag gaa	1159
Arg Ile His Pro Ala Ala Pro Leu Leu Ile Pro Arg Lys Val Glu Glu	
360 365 370 375	
gac gtt gag ttg tct acc tat att att cca aag gat tca caa gtt cta	1207
Asp Val Glu Leu Ser Thr Tyr Ile Ile Pro Lys Asp Ser Gln Val Leu	
380 385 390	
gtg aac gta tgg gca att gga cgc aac tct gat cta tgg gaa aat cct	1255
Val Asn Val Trp Ala Ile Gly Arg Asn Ser Asp Leu Trp Glu Asn Pro	
395 400 405	
ttg gtc ttt aag cca gaa agg ttt tgg gag tca gaa ata gat atc cga	1303
Leu Val Phe Lys Pro Glu Arg Phe Trp Glu Ser Glu Ile Asp Ile Arg	

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410	415	420	
ggt cga gat ttt gaa ctc att cca ttt ggt gct ggt cga aga att tgc			1351
Gly Arg Asp Phe Glu Leu Ile Pro Phe Gly Ala Gly Arg Arg Ile Cys			
425	430	435	
cct gga ttg cct ttg gct atg agg atg att cca gta gca cta ggt tca			1399
Pro Gly Leu Pro Leu Ala Met Arg Met Ile Pro Val Ala Leu Gly Ser			
440	445	450	455
ttg cta aac tca ttt aat tgg aaa cta tat ggt gga att gca cct aaa			1447
Leu Leu Asn Ser Phe Asn Trp Lys Leu Tyr Gly Gly Ile Ala Pro Lys			
460	465	470	
gat ttg gac atg cag gaa aag ttt ggc att acc ttg gcg aaa gcc caa			1495
Asp Leu Asp Met Gln Glu Lys Phe Gly Ile Thr Leu Ala Lys Ala Gln			
475	480	485	
cct ctg cta gct atc cca act ccc ctg tag ctatagggat aaattaagtt			1545
Pro Leu Leu Ala Ile Pro Thr Pro Leu			
490	495		
gaggttttaa gttactagta gattctattg cagctatagg atttctttca ccatcacgta 1605			
tgctttaccg ttggatgatg gaaagaaata tctatagctt tgggtttggt tagtttgcac 1665			
ataaaaattg aatgaatgga ataccatgga gttataagaa ataataagac tatgattctt 1725			
accctacttg aacaatgaca tggctatttc ac 1757			
<210> SEQ ID NO 27			
<211> LENGTH: 18			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:			
oligonucleotide			
<400> SEQUENCE: 27			
tttttttttt ttttttta 18			
<210> SEQ ID NO 28			
<211> LENGTH: 18			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:			
oligonucleotide			
<400> SEQUENCE: 28			
tttttttttt tttttttc 18			
<210> SEQ ID NO 29			
<211> LENGTH: 18			
<212> TYPE: DNA			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence:			
oligonucleotide			
<400> SEQUENCE: 29			
tttttttttt tttttttg 18			
<210> SEQ ID NO 30			
<211> LENGTH: 7			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic			
peptide representing a conserved region in plant cytochrome p450			
sequences.			

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<400> SEQUENCE: 30

Trp Ala Ile Gly Arg Asp Pro  
 1 5

<210> SEQ ID NO 31  
 <211> LENGTH: 20  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
 oligonucleotide  
 <220> FEATURE:  
 <221> NAME/KEY: Modified Base  
 <222> LOCATION: (6)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified Base  
 <222> LOCATION: (9)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified Base  
 <222> LOCATION: (12)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified Base  
 <222> LOCATION: (15)  
 <223> OTHER INFORMATION: n is inosine

<400> SEQUENCE: 31

tgggcnatng gnmngaycc

20

<210> SEQ ID NO 32  
 <211> LENGTH: 6  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 peptide representing a conserved region in plant cytochrome p450  
 sequences.

<400> SEQUENCE: 32

Phe Arg Pro Glu Arg Phe  
 1 5

<210> SEQ ID NO 33  
 <211> LENGTH: 22  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
 oligonucleotide  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (11)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (14)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (20)  
 <223> OTHER INFORMATION: n is inosine

<400> SEQUENCE: 33

aggaattymg nccngarmgn tt

22

<210> SEQ ID NO 34  
 <211> LENGTH: 32  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence



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<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
      oligonucleotide
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (3)
<223> OTHER INFORMATION: 2853:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (9)
<223> OTHER INFORMATION: 2858:<223> n is inosine
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<221> NAME/KEY: Modified base
<222> LOCATION: (12)
<223> OTHER INFORMATION: 2863:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (15)
<223> OTHER INFORMATION: 2868:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (18)
<223> OTHER INFORMATION: 2874:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (21)
<223> OTHER INFORMATION: 2879:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (24)
<223> OTHER INFORMATION: 2884:<223> n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (30)
<223> OTHER INFORMATION: 2889:<223> n is inosine

<400> SEQUENCE: 34

```

```

ccnttyggng cnggnmgnmg natntgkscn gg

```

32

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<210> SEQ ID NO 35
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
      peptide representing a conserved region in plant cytochrome p450
      sequences.
<220> FEATURE:
<221> NAME/KEY: UNSURE
<222> LOCATION: (3)
<223> OTHER INFORMATION: Xaa can be any amino acid.

<400> SEQUENCE: 35

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```

Glu Phe Xaa Pro Glu Arg Phe
  1             5

```

```

<210> SEQ ID NO 36
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
      oligonucleotide
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (3)
<223> OTHER INFORMATION: n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (7)
<223> OTHER INFORMATION: n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (8)
<223> OTHER INFORMATION: n is inosine

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-continued

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<220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (9)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (12)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (15)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (18)  
 <223> OTHER INFORMATION: n is inosine  
  
 <400> SEQUENCE: 36  
  
 ganttynnnc cnganmgntt 20  
  
 <210> SEQ ID NO 37  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
     oligonucleotide  
  
 <400> SEQUENCE: 37  
  
 ccacacgagt agttttggca ttgacctt 28  
  
 <210> SEQ ID NO 38  
 <211> LENGTH: 25  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
     oligonucleotide  
  
 <400> SEQUENCE: 38  
  
 gtcttgaca tcacacttca atctg 25  
  
 <210> SEQ ID NO 39  
 <211> LENGTH: 17  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
     oligonucleotide  
  
 <400> SEQUENCE: 39  
  
 ccgaattccc ccccccc 17  
  
 <210> SEQ ID NO 40  
 <211> LENGTH: 32  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence:  
     oligonucleotide  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (3)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (9)  
 <223> OTHER INFORMATION: n is inosine  
 <220> FEATURE:  
 <221> NAME/KEY: Modified base  
 <222> LOCATION: (12)

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<223> OTHER INFORMATION: n is inosine
<220> FEATURE:
<221> NAME/KEY: Modified base
<222> LOCATION: (18)
<223> OTHER INFORMATION: n is inosine
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<223> OTHER INFORMATION: n is inosine
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<221> NAME/KEY: Modified base
<222> LOCATION: (24)
<223> OTHER INFORMATION: n is inosine
<220> FEATURE:
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<223> OTHER INFORMATION: n is inosine

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<400> SEQUENCE: 40

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ccnggrcana tckyytncc ngcnccraan gg

```

32

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<210> SEQ ID NO 41
<211> LENGTH: 496
<212> TYPE: PRT
<213> ORGANISM: Petunia sp.

```

```

<400> SEQUENCE: 41

```

```

Met Asp Tyr Val Asn Ile Leu Leu Gly Leu Phe Phe Thr Trp Phe Leu
 1             5             10             15

Val Asn Gly Leu Met Ser Leu Arg Arg Arg Lys Ile Ser Lys Lys Leu
          20             25             30

Pro Pro Gly Pro Phe Pro Leu Pro Ile Ile Gly Asn Leu His Leu Leu
          35             40             45

Gly Asn His Pro His Lys Ser Leu Ala Gln Leu Ala Lys Ile His Gly
 50             55             60

Pro Ile Met Asn Leu Lys Leu Gly Gln Leu Asn Thr Val Val Ile Ser
 65             70             75             80

Ser Ser Val Val Ala Arg Glu Val Leu Gln Lys Gln Asp Leu Thr Phe
          85             90             95

Ser Asn Arg Phe Val Pro Asp Val Val His Val Arg Asn His Ser Asp
          100            105            110

Phe Ser Val Val Trp Leu Pro Val Asn Ser Arg Trp Lys Thr Leu Arg
          115            120            125

Lys Ile Met Asn Ser Ser Ile Phe Ser Gly Asn Lys Leu Asp Gly Asn
          130            135            140

Gln His Leu Arg Ser Lys Lys Val Gln Glu Leu Ile Asp Tyr Cys Gln
          145            150            155            160

Lys Cys Ala Lys Asn Gly Glu Ala Val Asp Ile Gly Arg Ala Thr Phe
          165            170            175

Gly Thr Thr Leu Asn Leu Leu Ser Asn Thr Ile Phe Ser Lys Asp Leu
          180            185            190

Thr Asn Pro Phe Ser Asp Ser Ala Lys Glu Phe Lys Glu Leu Val Trp
          195            200            205

Asn Ile Met Val Glu Ala Gly Lys Pro Asn Leu Val Asp Tyr Phe Pro
          210            215            220

Phe Leu Glu Lys Ile Asp Pro Gln Gly Ile Lys Arg Arg Met Thr Asn
          225            230            235            240

Asn Phe Thr Lys Phe Leu Gly Leu Ile Ser Gly Leu Ile Asp Asp Arg
          245            250            255

Leu Lys Glu Arg Asn Leu Arg Asp Asn Ala Asn Ile Asp Val Leu Asp

```

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260					265					270					
Ala	Leu	Leu	Asn	Ile	Ser	Gln	Glu	Asn	Pro	Glu	Glu	Ile	Asp	Arg	Asn
	275						280					285			
Gln	Ile	Glu	Gln	Leu	Cys	Leu	Asp	Leu	Phe	Ala	Ala	Gly	Thr	Asp	Thr
	290					295					300				
Thr	Ser	Asn	Thr	Leu	Glu	Trp	Ala	Met	Ala	Glu	Leu	Leu	Gln	Asn	Pro
305					310					315					320
His	Thr	Leu	Gln	Lys	Ala	Gln	Glu	Glu	Leu	Ala	Gln	Val	Ile	Gly	Lys
				325					330					335	
Gly	Lys	Gln	Val	Glu	Glu	Ala	Asp	Val	Gly	Arg	Leu	Pro	Tyr	Leu	Arg
			340					345					350		
Cys	Ile	Val	Lys	Glu	Thr	Leu	Arg	Ile	His	Pro	Ala	Ala	Pro	Leu	Leu
		355					360					365			
Ile	Pro	Arg	Lys	Val	Glu	Glu	Asp	Val	Glu	Leu	Ser	Thr	Tyr	Ile	Ile
	370						375				380				
Pro	Lys	Asp	Ser	Gln	Val	Leu	Val	Asn	Val	Trp	Ala	Ile	Gly	Arg	Asn
385				390						395					400
Ser	Asp	Leu	Trp	Glu	Asn	Pro	Leu	Val	Phe	Lys	Pro	Glu	Arg	Phe	Trp
				405					410					415	
Glu	Ser	Glu	Ile	Asp	Ile	Arg	Gly	Arg	Asp	Phe	Glu	Leu	Ile	Pro	Phe
			420					425					430		
Gly	Ala	Gly	Arg	Arg	Ile	Cys	Pro	Gly	Leu	Pro	Leu	Ala	Met	Arg	Met
		435					440					445			
Ile	Pro	Val	Ala	Leu	Gly	Ser	Leu	Leu	Asn	Ser	Phe	Asn	Trp	Lys	Leu
	450					455					460				
Tyr	Gly	Gly	Ile	Ala	Pro	Lys	Asp	Leu	Asp	Met	Gln	Glu	Lys	Phe	Gly
465				470					475					480	
Ile	Thr	Leu	Ala	Lys	Ala	Gln	Pro	Leu	Leu	Ala	Ile	Pro	Thr	Pro	Leu
				485					490					495	

&lt;210&gt; SEQ ID NO 42

&lt;211&gt; LENGTH: 513

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;400&gt; SEQUENCE: 42

Met	Ala	Thr	Leu	Phe	Leu	Thr	Ile	Leu	Leu	Ala	Thr	Val	Leu	Phe	Leu
1				5					10					15	
Ile	Leu	Arg	Ile	Phe	Ser	His	Arg	Arg	Asn	Arg	Ser	His	Asn	Asn	Arg
			20					25					30		
Leu	Pro	Pro	Gly	Pro	Asn	Pro	Trp	Pro	Ile	Ile	Gly	Asn	Leu	Pro	His
		35					40					45			
Met	Gly	Thr	Lys	Pro	His	Arg	Thr	Leu	Ser	Ala	Met	Val	Thr	Thr	Tyr
	50					55					60				
Gly	Pro	Ile	Leu	His	Leu	Arg	Leu	Gly	Phe	Val	Asp	Val	Val	Val	Ala
	65				70				75						80
Ala	Ser	Lys	Ser	Val	Ala	Glu	Gln	Phe	Leu	Lys	Ile	His	Asp	Ala	Asn
				85					90					95	
Phe	Ala	Ser	Arg	Pro	Pro	Asn	Ser	Gly	Ala	Lys	His	Met	Ala	Tyr	Asn
			100					105					110		
Tyr	Gln	Asp	Leu	Val	Phe	Ala	Pro	Tyr	Gly	His	Arg	Trp	Arg	Leu	Leu
		115					120						125		
Arg	Lys	Ile	Ser	Ser	Val	His	Leu	Phe	Ser	Ala	Lys	Ala	Leu	Glu	Asp
	130					135					140				

-continued

Phe	Lys	His	Val	Arg	Gln	Glu	Glu	Val	Gly	Thr	Leu	Thr	Arg	Glu	Leu
145					150					155					160
Val	Arg	Val	Gly	Thr	Lys	Pro	Val	Asn	Leu	Gly	Gln	Leu	Val	Asn	Met
				165					170					175	
Cys	Val	Val	Asn	Ala	Leu	Gly	Arg	Glu	Met	Ile	Gly	Arg	Arg	Leu	Phe
			180					185					190		
Gly	Ala	Asp	Ala	Asp	His	Lys	Ala	Asp	Glu	Phe	Arg	Ser	Met	Val	Thr
		195					200					205			
Glu	Met	Met	Ala	Leu	Ala	Gly	Val	Phe	Asn	Ile	Gly	Asp	Phe	Val	Pro
	210					215					220				
Ser	Leu	Asp	Trp	Leu	Asp	Leu	Gln	Gly	Val	Ala	Gly	Lys	Met	Lys	Arg
225					230					235					240
Leu	His	Lys	Arg	Phe	Asp	Ala	Phe	Leu	Ser	Ser	Ile	Leu	Lys	Glu	His
				245					250					255	
Glu	Met	Asn	Gly	Gln	Asp	Gln	Lys	His	Thr	Asp	Met	Leu	Ser	Thr	Leu
			260					265					270		
Ile	Ser	Leu	Lys	Gly	Thr	Asp	Leu	Asp	Gly	Asp	Gly	Gly	Ser	Leu	Thr
		275					280					285			
Asp	Thr	Glu	Ile	Lys	Ala	Leu	Leu	Leu	Asn	Met	Phe	Thr	Ala	Gly	Thr
	290					295					300				
Asp	Thr	Ser	Ala	Ser	Thr	Val	Asp	Trp	Ala	Ile	Ala	Glu	Leu	Ile	Arg
305					310					315					320
His	Pro	Asp	Ile	Met	Val	Lys	Ala	Gln	Glu	Glu	Leu	Asp	Ile	Val	Val
				325					330					335	
Gly	Arg	Asp	Arg	Pro	Val	Asn	Glu	Ser	Asp	Ile	Ala	Gln	Leu	Pro	Tyr
			340					345					350		
Leu	Gln	Ala	Val	Ile	Lys	Glu	Asn	Phe	Arg	Leu	His	Pro	Pro	Thr	Pro
		355					360					365			
Leu	Ser	Leu	Pro	His	Ile	Ala	Ser	Glu	Ser	Cys	Glu	Ile	Asn	Gly	Tyr
	370					375					380				
His	Ile	Pro	Lys	Gly	Ser	Thr	Leu	Leu	Thr	Asn	Ile	Trp	Ala	Ile	Ala
385					390					395					400
Arg	Asp	Pro	Asp	Gln	Trp	Ser	Asp	Pro	Leu	Ala	Phe	Lys	Pro	Glu	Arg
				405					410					415	
Phe	Leu	Pro	Gly	Gly	Glu	Lys	Ser	Gly	Val	Asp	Val	Lys	Gly	Ser	Asp
			420					425					430		
Phe	Glu	Leu	Ile	Pro	Phe	Gly	Ala	Gly	Arg	Arg	Ile	Cys	Ala	Gly	Leu
	435						440					445			
Ser	Leu	Gly	Leu	Arg	Thr	Ile	Gln	Phe	Leu	Thr	Ala	Thr	Leu	Val	Gln
	450					455					460				
Gly	Phe	Asp	Trp	Glu	Leu	Ala	Gly	Gly	Val	Thr	Pro	Glu	Lys	Leu	Asn
465					470					475					480
Met	Glu	Glu	Ser	Tyr	Gly	Leu	Thr	Leu	Gln	Arg	Ala	Val	Pro	Leu	Val
				485					490					495	
Val	His	Pro	Lys	Pro	Arg	Leu	Ala	Pro	Asn	Val	Tyr	Gly	Leu	Gly	Ser
			500					505					510		

Gly

<210> SEQ ID NO 43  
 <211> LENGTH: 7  
 <212> TYPE: PRT  
 <213> ORGANISM: Arabidopsis thaliana  
  
 <400> SEQUENCE: 43





What is claimed is:

1. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO:1.
2. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 3.
3. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 5.
4. An isolated nucleic acid molecule consisting of the nucleotide sequence as set forth in SEQ ID NO: 7.
5. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 9.
6. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 14.
7. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 16.
8. An isolated nucleic acid molecule comprising the nucleotide sequence as set forth in SEQ ID NO: 18.
9. An isolated nucleic acid molecule consisting of the nucleotide sequence as set forth in SEQ ID NO: 20.
10. An isolated nucleic acid molecule consisting of the nucleotide sequence as set forth in SEQ ID NO: 22.
11. An isolated nucleic acid molecule consisting of the nucleotide sequence as set forth in SEQ ID NO: 24.
12. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 2, or the complement of said nucleic acid molecule.
13. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 4, or the complement of said nucleic acid molecule.
14. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 6, or the complement of said nucleic acid molecule.
15. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 8, or the complement of said nucleic acid molecule.
16. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 10 or SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13, or the complement of said nucleic acid molecule.
17. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 15, or the complement of said nucleic acid molecule.
18. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 17, or the complement of said nucleic acid molecule.
19. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 19, or the complement of said nucleic acid molecule.
20. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 21, or the complement of said nucleic acid molecule.

21. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 23, or the complement of said nucleic acid molecule.
22. An isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO: 25, or the complement of said nucleic acid molecule.
23. A DNA construct capable of reducing expression of an endogenous gene encoding a flavonoid 3'-hydroxylase in a plant, said DNA construct comprising a nucleotide sequence selected from the group consisting of:
  - (i) a nucleotide sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 19; and
  - (ii) a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, and nucleotides 1478 to 3897 of SEQ ID NO: 9.
24. A method for producing a transgenic plant which synthesizes a flavonoid 3'-hydroxylase, said method comprising stably transforming a cell of a plant with the nucleic acid molecule according to any one of claims 1-3, 5-8 and 13-19 to produce a transformed cell, regenerating a transgenic plant from the transformed cell, and growing said transgenic plant wherein the nucleic acid molecule is expressed.
25. The method according to claim 24 wherein said plant is selected from the group consisting of petunia, carnation, chrysanthemum, rose, snapdragon, tobacco, cornflower, pelargonium, lisianthus, gerbera, apple, iris, lily, African violet and morning glory.
26. A transgenic plant having tissue exhibiting altered colour, said transgenic plant comprising a nucleic acid molecule selected from the group consisting of:
  - (i) a nucleotide sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 15, SEQ ID NO: 17, and SEQ ID NO: 19; and
  - (ii) a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, and nucleotides 1478 to 3897 of SEQ ID NO: 9.
27. A transgenic cut flower from the transgenic plant according to claim 26.
28. A transgenic seed from the transgenic plant according to claim 26.
29. A transgenic fruit from the transgenic plant according to claim 26.
30. A transgenic leaf from the transgenic plant according to claim 26.

\* \* \* \* \*



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 6,774,285 B1  
DATED : December 21, 2004  
INVENTOR(S) : F. Brugliera et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,  
Item [73], Assignee, "**Florigene Limited**" should read -- **International Flower Developments Pty Ltd.** --

Signed and Sealed this

Twenty-second Day of February, 2005

A handwritten signature in black ink on a dotted background. The signature reads "Jon W. Dudas" in a cursive style.

JON W. DUDAS

*Director of the United States Patent and Trademark Office*